An Exploratory Analysis of Regional Assessment Data in Support of Nutrient Criteria Development for EPA Regions 5, 7, and 8

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Executive summary

EPA recognizes the importance of nutrient criteria in protecting designated uses from eutrophication effects associated with phosphorus and nitrogen and has worked with states over the past 12 years to assist them in developing nutrient criteria. Towards that end, EPA has provided states and tribes with technical guidance to assess nutrient impairment and develop ecoregion-specific criteria. EPA published eco-regional criteria recommendations in 2000-2001 based on a frequency distribution approach meant to approximate reference condition concentrations. EPA also published recommendations in 2000 on scientifically defensible empirical approaches for setting numeric criteria. In November 2010, EPA elaborated on one of these empirical approaches in its publication, "Using Stressor-response Relationships to Derive Numeric Nutrient Criteria."

In developing nutrient criteria based on stressor-response analyses, States generally have relied on data from only within their boundaries. Using data from only within a state's boundaries works well where a significant portion of the state is relatively un-disturbed. In some locations, such as the central part of the United States, significant human-caused nutrient-related disturbance is widespread, and analysis of nutrient-related data within a state may not always show a consistent, strong relationship between nutrient enrichment and biological condition. In locations of significant nutrient-related disturbance, other parameters compete with nutrients in terms of impacting biology or otherwise confounding the identification of nutrient-biology relationships. High sediment loads can lead to light limitation, making the streams unresponsive to nutrients. Intermittent toxicity to plants and algae due to agricultural chemicals could also mask expected responses in Illinois streams. Nutrient concentrations may be above levels at which biological response would normally occur; subsequently, analysis may not observe much response as nutrient concentrations change. Where such conditions exist across most of a state, strong biological response to nutrients may not consistently be observed if using data from only within a state.

The purpose of this project was to identify nutrient response relationships from a cross-regional data set that could be used by individual states across the data analysis area. The analysis considers nutrient and biotic assemblage (macroinvertebrates) data across the Plains, Corn Belt, and Upper Midwest Regions from EPA's Wadeable Streams Assessment Survey. The analysis results herein suggest that data for large parts of EPA Regions 5, 7, and 8 could be aggregated for purposes of nutrient response threshold analysis, based on similarities in expected biological community.

This report should be considered as an exploratory effort in identifying types of analyses and stressor-response relationships that could be pursued in subsequent analyses. Given that the analysis results in this report tend to be consistent with the growing body of research on nutrient response thresholds, the report results could also be used as a line(s) of evidence for states and tribes in developing nutrient criteria.

Disclaimer

The analyses and opinions expressed in this report are the author's and do not reflect the policy of the U.S. Environmental Protection Agency, the Office of Research and Development, or the Mid-Continent Ecology Division.

Background

In April 2010, EPA staff at the Mid-Continent Ecology Division in Duluth, MN were asked by EPA Region 5 to analyze existing datasets to determine if they might be useful for setting nutrient criteria for Region 5 states (Ohio, Illinois, Indiana, Michigan, Wisconsin, and Minnesota). At that time, the only available dataset was from the 2004 Wadeable Streams Assessment (WSA, USEPA 2006). Analysis of additional datasets may be undertaken as they become available.

Purpose

The purpose of this report was to address the following questions:

- 1) Determine if there is a contiguous region of the central United States across which biological expectations are similar. Specifically,
 - a. Determine whether the sampled macroinvertebrate assemblages are dissimilar among WSA aggregated or level 3 ecoregions.
 - b. Determine whether the assemblages in the three level 3 ecoregions that constitute the Corn Belt Plains are dissimilar from assemblages elsewhere in the Midwest.
- 2) Conduct exploratory analyses across the central United States to identify candidate nutrient threshold values (CTVs) based on multiple analysis methods that could be used by states and tribes as lines of evidence in nutrient criteria development.
- 3) Determine how the thresholds from the analyses compare to published thresholds.
- 4) Based on the exploratory analyses, identify nutrient response relationships and types of analyses that merit additional evaluation.

Data and analyses

The data used in this analysis were extracted from the WSA dataset (http://www.epa.gov/owow/streamsurvey/web_data.html). Files containing stream water chemistry data, site information data, benthos metrics, benthos counts (genus level), and physical habitat data were downloaded for all WSA sites. National Land Cover Database (NLCD) 2001 data were compiled for all sites in the Plains and Upper Midwest (Fig. 1). Plains and Upper Midwest are defined for WSA as shown in Fig. 2. The Plains and Upper Midwest includes the following aggregate ecoregions: Upper Midwest, Temperate Plains, Southern Plains, and Northern Plains). Level 3 ecoregions in this report are from Omernik (1987). Land cover data does not include watershed area in Canada. Records without total N or total P data were deleted. Preliminary analysis identified one site with only three macroinvertebrate taxa in the sample, which was deleted. Site revisits were excluded from this analysis. The final dataset had WSA data for 1371 sites with NLCD data for 327 Plains/Upper Midwest sites (Fig. 1). Analyses were

conducted using SAS System for Windows v.9.1 statistical software, Sigmaplot for Windows v.11 graphics and statistical software, and PRIMER v.6 software for multivariate analysis. Information on WSA sample design and field methods are available online: http://water.epa.gov/type/rsl/monitoring/streamsurvey/index.cfm

The analysis in this report includes classification of Plains and Upper Midwest WSA sites (objective 1) and derivation of candidate threshold values (objectives 2-3). Nine different methods (Table 1) were used to derive candidate threshold values (CTV). Graphical, regression and multivariate methods were used to examine the data. The following analyses were performed:

Analysis 1:

ANOSIM (Primer), a multivariate ANOVA analog, was used to test if sites from different groups had similar assemblages using the R-statistic. When R is large (>>0.2) the assemblages are relatively dissimilar (i.e., there is a strong group effect); when R is small (<<0.2) there is high overlap in assemblages between groups. ANOSIM was used to compare WSA aggregated ecoregions (Fig. 2), level 3 ecoregions (if $n \ge 5$ sites), Plains/Upper Midwest sites in the Corn Belt plains and not in the Corn Belt plains, and between groups of sites based on natural variation (northern and southern streams, steep and flat streams, streams with large and small watersheds, and streams with fine and coarse dominant substrate). Based on the ANOSIM results, sites in the Plains/Upper Midwest aggregated ecoregions (Upper Midwest, Southern Plains, Northern Plains, Temperate Plains) were retained for further analyses (n = 327).

Analysis 2:

Means and selected percentiles of nutrient concentrations were computed for all Plains/Upper Midwest sites, for WSA aggregated ecoregions, and for sites in the Corn Belt Plans (CBP) and sites not in then CBP, reference and non-reference sites, and sites in Illinois or Indiana and sites not in Illinois or Indiana (CTV methods 1 and 2, Table 1). Reference sites were identified in the WSA data site. For an explanation of the WSA reference approach, go to http://water.epa.gov/type/rsl/monitoring/streamsurvey/upload/2007_5_9_streamsurvey_04_chap_1_5-2-07.pdf.

Analysis 3:

Principal components analysis (PCA, PRIMER) was used to generate a human disturbance gradient (PC 1) based on the percent of the site's watershed in row crops, pasture or hay, development, forest, or wetlands, and riparian disturbance and predict "background" nutrient concentrations. Human population density, road density, and canopy openness were not included in the PCA because of missing values in the source data. The PCA is an alternative approach to the multiple linear regression approach described below (Analysis 4) that allows the inclusion of natural land covers (forest, wetland) in the gradient. Simple and piecewise three-segment linear regression (Sigmaplot) was used to predict the "background" nutrient concentration (y) at a site with the lowest value for PC 1 (CTV method 4, Table 1).

Analysis 4:

Multiple linear regression (SAS) was used to predict the "background" nutrient concentration (y) at sites with all human disturbances (x) set to background concentration (i.e., the y-intercept)

(CTV method 3, Table 1). Human disturbance variables included the percent of the site's watershed in row crops, pasture or hay, and development, the human population of the watershed, the road density, the riparian condition, and the percent riparian canopy openness (100 – percent canopy coverage). Mallow's *Cp* statistic was used to select the best models without over-fitting (http://www.statistics4u.info/fundstat_eng/cc_varsel_mallowscp.html).

Analysis 5:

Multiple linear regression was used to predict macroinvertebrate metric values at all sites using natural variation variables (latitude, longitude, mean stream wetted width, mean channel slope, dominant substrate particle size, watershed area, and annual precipitation). Regression residuals, representing variation in metrics not explained by natural variables, were retained for further analysis.

Analysis 6:

Piecewise linear regression (Sigmaplot) was used to determine "breakpoints" in relationships between twenty-four non-redundant (r_s < 0.85) "responsive" macroinvertebrate assemblage metrics (y) and log-transformed nutrient concentrations (x). Responsive metrics were those that were at least weakly rank correlated ($r_s \ge 0.25$) with at least one nutrient. Other methods for breakpoint (or change point) analysis are available, but the piecewise approach is straightforward, relatively easy to implement, and the results are amenable to comparison with hypothesized responses.

The choice of a three-segment regression was based on a hypothetical three-segment response to increasing nutrient concentration (Fig. 3A and 3B). In this idealized response, metrics values are all high (plot A) or all low (for metrics that increase with nutrients, plot B) until a "response threshold" (*Rt*) is reached. Metric values then decrease (or increase) to a secondary threshold (*St*) beyond which they do not change much. The midpoint of this response is at *Rm*, which is less protective than *Rt* because some decrease in the metric has already occurred. *Rm* represents the middle of the range over which the metric responds most strongly to nutrients. *St* (as in Fig. 3A and 3B) is undesirable as a threshold since it may mark the beginning of an alternative stable state in the macroinvertebrate fauna from which ecological recovery may be difficult (Dodds et al 2010). The dashed lines in Fig 3 indicate that there can be some deviation from a perfectly stable metric in the sub-threshold range.

In an alternative three-segment response (Fig 3C and 3D), metrics values decline from the minimum observed nutrient concentration, Mn, to a secondary threshold at St. The biological interpretation of this response is not clear since there is no response threshold. The midpoint of this response at Rm is not necessarily protective because the true value of Rt may occur below the observed range of nutrient concentrations.

If there were no significant breakpoints in the three-segment model, a two-segment piecewise regression was fit to the data. The interpretation of the hypothesized two-segment response (Fig. 3E and 3F) is similar to the three-segment response (Fig 3A and 3B) except that there is no secondary threshold (St becomes Mx, or the maximum observed value). The interpretation of the

response depicted in Fig. 3G and 3H is analogous to the three-segment response depicted in Fig. 3C and 3D.

If there were no significant breakpoints in the two-segment model, a simple linear regression was fit to the data.

The interpretation of the biological meaning of breakpoints is somewhat subjective. Where appropriate, the first breakpoint with increasing nutrient concentration (Rt) was defined as the candidate threshold value (CTV). Rt is a protective threshold that conforms to the hypothesized response. In some cases, Rm was selected even when the response resembled the hypothesized response (Figs. 3A, 3B, 3E, or 3F) because of high variability or sparse data when Rt occurred at the extreme of the measured nutrient data. In other cases, the only significant breakpoint was detected at such an extreme value that the relationship was considered linear and no threshold was inferred.

A breakpoint in a metric response to a nutrient is not necessarily biologically relevant or appropriate for threshold determination. Breakpoint analysis can be potentially misleading especially if applied systematically without an underlying biological model. In this report, every breakpoint was individually examined with respect to the hypothesized responses and the variability and density of data near the breakpoint. The rationale for each CTV based on a breakpoint is documented in an appendix to this report.

The piecewise regression analysis was conducted on raw metric values (CTV method 5, Table 1), on the regression residuals described above (CTV method 6, Table 1), and on raw metric values for each of two stream groups: streams with coarse substrate (\geq 1 mm), and streams with fine substrate (<1 mm) (CTV method 7, Table 1).

CTV values derived from biotic responses to nutrient concentrations were plotted against the r^2 value of the associated simple linear or breakpoint regression to determine if CTV converges on a threshold value as predictive power increases.

Analysis 7:

Interpolation of simple linear regressions of raw biotic data (y) with nutrient concentrations (x) were used to derive reference nutrient concentration for each biotic metric (CTV method 8, Table 1). Reference expectations (the value for the biotic metric at reference sites) were based on a percentile of the reference population as defined for WSA. The same analysis was separately conducted for streams with fine and coarse substrate. (CTV method 9, Table 1). CTV values derived from biotic responses to nutrient concentrations were plotted against the r^2 value of the associated simple linear or breakpoint regression to determine if CTV converges on a threshold value as predictive power increases.

Analysis 8:

From percentage frequency distributions, the percent of Plains/Upper Midwest streams and the percent of streams in Indiana and Illinois with nutrient concentrations above a CTV were determined.

Analysis 9:

CTVs derived from the preceding analyses were compared to published threshold values for the Plains and Upper Midwest.

Findings

Spatial variation in macroinvertebrate assemblages (Analysis 1)

Many comparisons between WSA aggregated ecoregions were significant (Table 2). However, none of the comparisons with a strong group effect included comparisons between WSA aggregated ecoregions in the Plains and Upper Midwest. Only one comparison (Upper Midwest compared to the Southern Plains) had a moderate groups effect (R = 0.30). The other comparisons between Plains/Upper Midwest aggregated ecoregions were weak or negligible. Assemblages in streams in the Corn Belt Plains were similar to assemblages in non-Corn Belt Plains streams. Assemblages in streams in Illinois and Indiana were similar to assemblages in other states.

Many comparisons among assemblages for level 3 Plains ecoregions were significant (39%, Table 3). The strongest distinctions (highest *R* statistics) were generally for widely separated ecoregions. Only three significant comparisons were for ecoregions that were contiguous (i.e., shared a border). The large number of significant comparisons between relatively small regions across a vast area is not surprising, but the results must be interpreted with caution because of small sample sizes.

Nutrient concentrations (Analysis 2)

Mean total N concentration was highest in the Temperate Plains (5252 ug/L) and lowest (<1350 ug/L) in the Northern and Southern Plains (Table 4). Mean total P concentration was highest in the Northern and Temperate Plains aggregated ecoregions (≥240 ug/L) and lowest in the UMW aggregated ecoregion (85 ug/L).

Mean total N concentration was higher in the Corn Belt Plains (8539 ug/L) than elsewhere in the Plains/Upper Midwest (1674 ug/L). Mean total P concentration was similar in both groups (212 and 234 ug/L, Table 4).

Sites identified as reference in the WSA dataset had much lower mean total N and total P concentrations than other non-reference sites (Table 4). Sites in Illinois and Indiana had a higher mean total N concentration than other plains sites. Mean total P concentration was the same at sites in Illinois and Indiana as sites elsewhere in the Plains/Upper Midwest.

For level 3 ecoregions with ≥ 8 sites, total N concentration was highest in the Central and Western Corn Belt Plains (>9600 ug/L, Table 5), and lowest in the Northern Lakes and Forests and North Central Hardwoods (<600 ug/L). Total P concentration (when $n \geq 8$) was highest in the Northwestern Glaciated Plains and Central Great Plains (>430 ug/L) and was lowest in Northern Lakes and Forests (42 ug/L).

Predicting "background nutrient" concentration from PCA (Analysis 3)

Human disturbance varied among aggregated WSA ecoregions (Table 6). Percent of the watershed in row crops was highest in the Temperate Plains (60%) and lowest in the Northern and Southern Plains (18%). Watersheds in WSA aggregated ecoregions were 5-7 percent developed except in the Northern Plains, which was less developed (2%). Percent forest and wetland were much higher in the Upper Midwest (57% combined) than other WSA aggregated ecoregions (≤20% combined). Population density and road density followed a similar pattern. Riparian disturbance and canopy openness were lower at Upper Midwest sites than at sites elsewhere in the Plains/Upper Midwest.

Human disturbance was consistently greater in the Corn Belt Plains and non-reference sites than at non Corn Belt Plains and reference sites (Table 6).

The first principal component explained 35% of the variation in the ordination and was primarily a gradient from relatively undisturbed Upper Midwest sites with a high percentage of forest and wetlands in their watershed to sites (including virtually all Temperate Plains sites) with a relatively high percentage of row crops, development, and riparian disturbance (Fig. 4). The second principal component (24% of variance explained) separated Northern and Southern Plains sites with high riparian disturbance but low percentage of forest, wetland, pasture/hay, and development, from the other WSA aggregated ecoregions.

Predicting background nutrient concentration from human disturbance (Analysis 4)

A multiple regression model that included percent row crops, percent pasture/hay, road density, and canopy openness predicted a background total N concentration at a Plains/Upper Midwest site of 306 ug/L ($r^2 = 0.54$, Table 7). A weaker model ($r^2 = 0.17$) that included percent row crops, percent pasture/hay, population density, riparian disturbance, and canopy openness predicted a background total P concentration of 26 ug/L.

Predicted background total N concentration (Table 7) was highest at Northern Plains sites (526 ug/L) and lowest at Temperate Plains sites (288 ug/L). Background total P concentration could only be reliably predicted for the Southern Plains (23 ug/L) and Upper Midwest (19 ug/L).

Predicted background total N concentration at Corn Belt Plains sites was 333 ug/L, but could not be reliably predicted for total P. Predicted background total N and total P concentration for a site in Illinois or Indiana was 196 and 34 ug/L, respectively (Table 7).

A three-segment piecewise regression model explained 46% of the variation in total N concentration with PC 1 (Fig. 5A). This regression approach was used because there seemed to be different relationships between human disturbance (PC1) and total N for each of three parts of the disturbance range (the range of mostly Upper Midwest sites, the range of mostly Northern + Southern Plains sites, and the range of mostly Temperate Plains sites). At sites with minimal disturbance (lowest value for PC 1), which were Upper Midwest sites with a high percentage of forest and wetland (Fig 5), the predicted background total N concentration was 436 ug/L. A

linear regression model explained 19% of the variation in total P concentration with PC 1. The predicted background total P concentration was 16 ug/L at sites with minimum human disturbance (Fig. 5B).

Macroinvertebrate metric relationship to natural variation (Analysis 5)

All natural factors had a significant effect on at least some macroinvertebrate metrics (Appendix 1). Substrate had the highest (based on F-statistics) and most consistent significant effect on metric values, followed by latitude. Mean values for natural variables are given in Appendix 2.

Substrate was significantly rank correlated with longitude, latitude, mean slope, mean stream width, and precipitation (Appendix 3). Of the natural factors operating at a site, macroinvertebrate assemblages are likely to be most influenced by substrate characteristics.

Streams were divided into two substrate group for further analysis: streams with fine substrate (< 1 mm mean diameter) and streams with coarse substrate (≥1 mm mean diameter) (Appendix 4). Plots of macroinvertebrate metrics with substrate size (Fig. 6) show that the biologically significant substrate size cut-off probably varies among metrics (e.g., > 1 mm for HBI and TL89PTAX; < 1 mm for TL03PIND and EPT_RICH), but 1 mm (log-transformed value of 0 mm) is a reasonable approximation of the critical value.

Reference sites had a greater percentage of sites with coarse substrate (62%) than fine substrate (38%). For all other groups, more sites had fine substrate. Overall, 70 percent of sites had fine substrate. Mean diameter of substrate in streams in each substrate group are given in Appendix 3.

Results of the multiple regressions based on individual metrics (Appendix 1) are not corroborated by ANOSIM results (Table 2). Across all sites, assemblages in streams with fine substrates were not dissimilar to assemblages in streams with coarse substrates (R = 0.03). Dissimilarity between WSA aggregated ecoregions was greater than variation between natural groups, suggesting that at a regional scale, biogeographic variation (e.g., in species pool composition) accounts for more variation than local factors.

Candidate threshold values based on macroinvertebrate metric response to nutrients – breakpoint regression (Analysis 6)

Using breakpoint regression, a candidate threshold value (CTV) could be derived for the response of most metrics to nutrient concentration (Tables 8-11). Exceptions were when there was no significant breakpoint (the relationship was linear) or when breakpoints occurred near an extreme of the data and were judged unreliable (how the CTV was determined for each metric is documented in Appendix 5; data plotted in Appendix 6).

For the response of raw metrics to total N concentration, there was one extreme value (12,022 ug/L) for OLLEPTAX, which is based on a very tolerant group, the Oligochaeta (See Appendix 10 for explanation of metric names). Median CTV for all metrics was 379 ug/L total N (from Table 8).

The same analysis conducted using the regression residuals (from models summarized in Table 8) instead of the raw metrics produced generally similar results. Although a few more metrics lacked or had unreliable breakpoints, the median CTV (388 ug/L) was similar to the median CTV for raw metrics (379 ug/L). Median strength of models based on r^2 was similar for raw metrics (0.10) and regression residuals (0.11).

Plotting CTV for total N against the r^2 value for the piecewise regression (Fig. 7A) shows that as r^2 increases, the CTV converges on a value between 100 and 1000 ug/L for both the raw metrics and the CTVs based on the regression residuals.

For the response of raw metrics to total P, there were two extreme CTVs, 371 and 561 ug/L, for HPRIME and HBI, respectively. HBI is a positive metric that increases with increasing pollution and the high CTV may reflect the response of the most tolerant organisms in the sample. Median CTV for all metrics was 17 ug/L total P (Table 9).

The same analysis conducted using the regression residuals (Table 9) instead of the raw metrics produced generally similar results. The median CTV (20 ug/L total P) was similar to the median CTV for raw metrics (17 ug/L). Median strength of models based on r^2 was slightly higher for raw metrics (0.11) than for models based on regression residuals (0.07).

Plotting CTV for total P against the r^2 value for the piecewise regression (Fig. 7B) shows that as model fit increases, variation in the CTVs decreases for both the raw metrics and the models based on the regression residuals. CTV values associated with higher r^2 values were generally within a range of 10-100 ug/L total P.

Separate analysis for streams with fine and coarse substrate had only a small effect on the results for total N (Table 10). Median r^2 and CTV values for total N in streams with fine substrate were about the same as for all streams combined. (Table 8 and 10; data plotted in Appendix 7). Median r^2 and CTV values for total N in streams with coarse substrate were also similar to all streams combined.

Classifying the streams by substrate size had a greater effect on results for total P (Table 9-11; data plotted in Appendix 5). Median r^2 for streams with fine substrates (0.08) were similar to all streams (0.11). However, median r^2 for streams with coarse substrates (0.20) was higher than the median r^2 for all streams (0.07). The reason for the stronger relationships for streams with coarse substrate is not known, but is probably related to the degree of impairment these streams. Reference sites, most of which have coarse substrate (Appendix 4), are steeper (Appendix 2), and more agricultural (Table 6). There were probably more relative unimpaired sites among coarse substrate streams allowing for better detection of a response by macroinvertebrates to nutrients.

Plotting CTV for total N and total P against the r^2 value for the piecewise regression (Fig. 8) shows that as model fit increases, variation in the CTVs decreases for both streams with fine and coarse substrates.

Candidate threshold values based on macroinvertebrate metric response to nutrients – interpolation of linear regression (Analysis 7)

Using interpolation of simple linear regression, a candidate threshold value (CTV) could be derived for the response of metrics to nutrient concentration (Tables 12-17). This method requires reliable reference expectations for each metric. For the WSA data, metric values for the worst reference sites (the 25th or 75th percentile of reference sites, depending on whether the metrics increased or decreased with increasing nutrients) were, in most cases, **worse** than metric values for the best non-reference sites (the 25th or 75th percentile of non-reference sites, depending on whether the metrics increased or decreased with increasing nutrients). Because of this, the reference expectation corresponding to the median metric value for the reference sites was use in the interpolations.

The CTVs for total N based on interpolation for all streams ranged from 2 to 5570 ug/L, with a median CTV or 347 ug/L (Table 12). Because of the weak relationships between nutrients and metrics ($r^2 \le 0.15$), the prediction intervals were very wide (Table 12, Appendix 8).

The CTVs for total P based on interpolation for all streams ranged from 2 to 436 ug/L, with a median CTV of 21 ug/L (Table 13). Because of the weak relationships between nutrients and metrics ($r^2 \le 0.20$), the prediction intervals were very wide (Table 13, Appendix 8).

Plotting CTVs against the r^2 value for the interpolations (Fig. 9) shows that as model fit increases, variation in the CTVs decreased. CTV values associated with higher r^2 values were generally with a range of 100 -1000 ug/L total N and 10-100 ug/L total P.

The CTVs for total N based on interpolation for streams with fine substrate ranged from 0 to 7369 ug/L, with a median CTV of 164 ug/L (Table 14, Appendix 9). The CTV values for total N based on interpolation for streams with coarse substrate ranged from 4 to 13701 ug/L, with a median CTV of 636 ug/L (Table 15, Appendix 9).

The CTVs for total P based on interpolation for streams with fine substrate ranged from <0 to 1461 ug/L, with a median CTV of 10 ug/l (Table 16). The CTV values for total P based on interpolation for streams with coarse substrate ranged from 6 to 294 ug/L, with a median CTV of 43 ug/L (Table 17).

Plotting CTVs against the r^2 value for the substrate-specific interpolations (Fig. 10) shows that as model fit increases, variation in the CTVs decreased. CTV values associated with higher r^2 values were generally with a range of 100 -1000 ug/L total N and 10-100 ug/L total P.

Regional assessment based on derived CTVs (Analysis 8)

Cumulative percentage distribution curves (Fig. 11) show the percentage of stream sites that would have a nutrient concentration above a particular value. The curves are based on single water grab samples at each site, so the percentages must be considered provisional. Across the range of criteria derived from WSA data in this report (about 100-1000 ug/L total M, and 10-100 ug/L total P), the percent of Plains/Upper Midwest streams exceeding the total N criteria ranges from 53 to 99%. The percent of streams in Indiana and Illinois exceeding the total N criteria

ranges from 72 to 100%. The percent of Plains/Upper Midwest streams exceeding the total P criteria ranges from 45 to 95%. The percent of streams in Indiana and Illinois exceeding the total N criteria ranges from 43 to 100%.

Comparisons with published criteria (Analysis 9)

The CTV range for total N and total P suggested by biotic response to nutrients (100 to 1000 ug/L total N and 10-100 ug/L total P) bounds the other CTVs derived from predictive models and percentiles of the population (Fig. 12). Most published threshold values fall in this same range. Exceptions include values reference-percentile based value of Herlihy and Sifneos (2008) which is based on reference sites in the Corn Belt Plains, and the population based percentile values of Robertson et al. 2001).

Major conclusions

- 1) Compared to dissimilarity between macroinvertebrate assemblages in super-regions and between all pairs of aggregated WSA ecoregions, dissimilarity between Plains/Upper Midwest aggregate ecoregions was low. Assemblages in streams in the Corn Belt Plains were not different from streams elsewhere in the Plains/Upper Midwest.
- 2) Among aggregated WSA ecoregions, mean total N was by far the highest in the Temperate Plains (5252 ug/L). Mean total P was highest in the Northern Plains (283 ug/L) and nearly as high in the Temperate Plains (240 ug/L).
- 3) Background nutrient concentration in a Plains/Upper Midwest wadeable stream predicted from linear regression of PC1 were 436 ug/L total N and 16 ug/L total P.
- 4) Background nutrient concentrations in a Plains/Upper Midwest wadeable stream predicted from multiple linear regression were 306 ug/L total N and 26 ug/L total P. Predicted background concentrations for Illinois and Indiana were lower for total N (196 ug/l) and slightly higher for total P (34 ug/L), but sample size was small, and these estimates lack precision. Percent row crops was a significant parameter in nearly all models.
- 5) Separate analysis of the macroinvertebrate response to nutrient concentration for streams with fine and coarse substrates resulted in stronger relationships for total P in streams with coarse substrate. Substrate-specific analysis could be used to adjust regional or state criteria.
- 6) The range of CTVs herein based on macroinvertebrate metric responses to nutrient concentration generally overlapped potential regional nutrient criteria derived by other methods both in the literature and from this study.

The determination of thresholds based on breakpoints in this report was, in some cases, partly subjective because of variability at the extremes of the data range and/or weak responses to nutrients for some metrics. Despite the subjectivity in determining the CTVs for each metric-nutrient combination, a weight of evidence-based threshold is possible because CTVs tend to

converge (variability decreases) as the strength of piecewise regression models increases (r^2 increases).

- 7) If states adopted nutrient criteria for the Plains/ Upper Midwest set at the low end of the range suggested by the CTVs presented here (100 ug/L total N and 10 ug/L total P), most stream sites (>95%) would exceed nutrient criteria. If states adopted nutrient criteria that were set at the upper end of the range suggested by the CTVs presented here (1000 ug/L total N and 100 ug/L total P), about half of the stream sites would exceed nutrient criteria.
- 8) In this exploratory analysis, most of the biotic responses to nutrient concentration were weak, with r^2 values mostly <0.2 which means that less than 20% of the variation in biotic metrics was explained by variation in nutrient concentration. It may be that most streams are already significantly impaired by nutrients beyond the range of conditions over which a response to nutrients can be detected. Natural and sampling variation also confounds biotic responses. Some of the variation can be attributed to data quality from the Wadeable Stream Assessment. Estimates of nutrient concentration are based on a single summer grab sample. Estimates of macroinvertebrate assemblage metrics are based on single summer composite sample. Summer base flow conditions may not reflect the strongest response of many macroinvertebrate assemblage metrics to nutrient stress.

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Table 1. Summary of methods used in this report to derive candidate threshold values (CTV) for total N and total P.

	Method	Groups
1	Percentile of the population	Multiple
2	Percentile of reference population	Multiple
3	Multiple linear regression predicting CTV from stressor variables	WSA Plains/Upper Midwest
4	Simple linear regression predicting CTV from PCA stressor gradient	WSA Plains/Upper Midwest
5	Piecewise regression of raw biotic data with nutrient concentration. CTVs based on breakpoint	WSA Plains/Upper Midwest
6	Piecewise regression of residual values with nutrient concentration. CTVs based on breakpoint	WSA Plains/Upper Midwest
7	Piecewise regression of raw biotic data with nutrient concentration data split by substrate size. CTV based on breakpoint	WSA Plains/Upper Midwest
8	Interpolation of CTVs for reference condition from linear regression of raw biotic data with nutrient concentrations.	WSA Plains/Upper Midwest
9	Interpolation of CTVs for reference condition from linear regression of raw biotic data with nutrient concentrations data split by substrate size	WSA Plains/Upper Midwest

Table 2. *R*-statistics for comparisons of macroinvertebrate assemblage composition (based on genera) between selected ecoregional and natural variation groups. High values of *R* indicate dissimilar assemblages. Highlighted comparisons are WAS aggregated Plains/Upper Midwest ecoregions. WMT = Western Mountains, CPL = Coastal Plain, SPL = Southern Plains, TPL = Temperate Plains, SAP = Southern Appalachians, NAP = Northern Appalachians, XER = Xeric, UMW = Upper Midwest, NPL = Northern Plains.

Groups	R	Interpretation
CPL, WMT	0.84	Strong group effect
TPL, WMT	0.75	Strong group effect
SPL, WMT	0.73	Strong group effect
WMT, NPL	0.72	Strong group effect
SAP, WMT	0.62	Strong group effect
UMW, WMT	0.55	Strong group effect
SAP, SPL	0.52	Strong group effect
CPL, XER	0.50	Strong group effect
NAP, SPL	0.47	Strong group effect
SAP, NPL	0.47	Strong group effect
NAP, WMT	0.44	Moderate group effect
NAP, NPL	0.44	Moderate group effect
SAP, XER	0.42	Moderate group effect
NAP, CPL	0.39	Moderate group effect
TPL, XER	0.38	Moderate group effect
TPL, NAP	0.37	Moderate group effect
SAP, CPL	0.34	Moderate group effect
WMT, XER	0.33	Moderate group effect
TPL, SAP	0.32	Moderate group effect
UMW, SPL	0.30	Moderate group effect
CPL, NPL	0.28	Moderate group effect
XER, NPL	0.27	Moderate group effect
SPL, XER	0.27	Moderate group effect
UMW, CPL	0.26	Weak group effect
UMW, NPL	0.26	Weak group effect
CPL, SPL	0.25	Weak group effect
TPL, SPL	0.23	Weak group effect
UMW, SAP	0.23	Weak group effect
TPL, CPL	0.20	Weak group effect
TPL, UMW	0.18	Negligible group effect
UMW, NAP	0.17	Negligible group effect
NAP, XER	0.17	Negligible group effect
SPL, NPL	0.16	Negligible group effect
UMW, XER	0.14	Negligible group effect
TPL, NPL	0.13	Negligible group effect
NAP, SAP	0.10	Negligible group effect
Indiana or Illinois, not Indiana or Illinois (Plains/Upper Midwest)	< 0.01	Group effect not significant
Corn Belt Plains, not Corn Belt Plains (Plains/Upper Midwest)	< 0.01	Group effect not significant
Aggregated WSA ecoregions (overall effect)	0.19	Weak group effect
		Group effect not significant

Steep streams ($\geq 1\%$), flat streams ($<1\%$) ²	0.03	Group effect not significant
Fine substrate (<1 mm); coarse substrate \geq 1 mm) ³	0.03	Group effect not significant
Northern streams (\geq 40 N), southern streams ($<$ 40N) ⁴	0.07	Group effect not significant

¹ Group means (95% CI): small, 35(31-39) km², large, 2903 (1918-3887) km² Group means (95% CI): steep, 1.5 (1.4-1.6) %, flat, 0.37 (0.33-0.40) % Group means (95% CI): fines, 0.10 (0.08-0.12) mm, coarse, 4.5 (2.5-5.9) mm ⁴ Group means (95% CI): north, 44.5 (44.2-44.8) dd, south, 37.4 (36.8-28.0) dd

Table 3. R statistics for significant comparisons of macroinvertebrate assemblage composition between level 3 ecoregions (i.e., groups) with n \geq 5. High values for R indicate dissimilar assemblages. 60 of 153 pairwise comparisons were significant. Highlighted comparisons are level 3 ecoregions that were contiguous with each other. Sample sizes in Table 5.

Groups	R
DRIFTLESS AREA, CENTRAL OLKAHOMA/TEXAS PLAINS	0.74
S. MICHIGAN/N. INDIANA DRIFT PLAINS, WESTERN HIGH PLAINS	0.71
DRIFTLESS AREA, NORTHERN GLACIATED PLAINS	0.64
SOUTHEASTERN WISCONSIN TILL PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.64
DRIFTLESS AREA, CENTRAL GREAT PLAINS	0.64
INTERIOR RIVER VALLEYS AND HILLS, WESTERN HIGH PLAINS	0.63
DRIFTLESS AREA, INTERIOR RIVER VALLEYS AND HILLS	0.63
DRIFTLESS AREA, LAKE AGASSIZ PLAIN	0.62
NORTH CENTRAL HARDWOOD FORESTS, NORTHERN GLACIATED PLAINS	0.61
DRIFTLESS AREA, CENTRAL IRREGULAR PLAINS	0.60
CENTRAL OLKAHOMA/TEXAS PLAINS, SOUTHEASTERN WISCONSIN TILL PLAINS	0.59
EASTERN CORN BELT PLAINS, WESTERN HIGH PLAINS	0.59
NORTH CENTRAL HARDWOOD FORESTS, CENTRAL OLKAHOMA/TEXAS PLAINS	0.57
WESTERN HIGH PLAINS, NORTHERN GLACIATED PLAINS	0.57
INTERIOR RIVER VALLEYS AND HILLS, NORTH CENTRAL HARDWOOD FORESTS	0.55
DRIFTLESS AREA, EASTERN CORN BELT PLAINS	0.54
NORTHERN LAKES AND FORESTS, SOUTHWESTERN TABLELANDS	0.52
NORTH CENTRAL HARDWOOD FORESTS, LAKE AGASSIZ PLAIN	0.51
DRIFTLESS AREA, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.50
NORTHERN LAKES AND FORESTS, WESTERN HIGH PLAINS	0.50
WESTERN HIGH PLAINS, LAKE AGASSIZ PLAIN	0.45
NORTHERN LAKES AND FORESTS, CENTRAL GREAT PLAINS	0.45
S. MICHIGAN/N. INDIANA DRIFT PLAINS, NORTHERN GLACIATED PLAINS	0.43
CENTRAL IRREGULAR PLAINS, NORTH CENTRAL HARDWOOD FORESTS	0.43
SOUTHEASTERN WISCONSIN TILL PLAINS, NORTHERN GLACIATED PLAINS	0.42
NORTHERN LAKES AND FORESTS, LAKE AGASSIZ PLAIN	0.42
NORTH CENTRAL HARDWOOD FORESTS, EASTERN CORN BELT PLAINS	0.41
CENTRAL GREAT PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.41
INTERIOR RIVER VALLEYS AND HILLS, SOUTHWESTERN TABLELANDS	0.39
EASTERN CORN BELT PLAINS, NORTHERN GLACIATED PLAINS	0.39
DRIFTLESS AREA, NORTHERN LAKES AND FORESTS	0.38
S. MICHIGAN/N. INDIANA DRIFT PLAINS, LAKE AGASSIZ PLAIN	0.38
CENTRAL OLKAHOMA/TEXAS PLAINS, EASTERN CORN BELT PLAINS	0.37
WESTERN CORN BELT PLAINS, NORTHWESTERN GLACIATED PLAINS	0.37
INTERIOR RIVER VALLEYS AND HILLS, NORTHWESTERN GLACIATED PLAINS	0.37
WESTERN CORN BELT PLAINS, SOUTHWESTERN TABLELANDS	0.37
INTERIOR RIVER VALLEYS AND HILLS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.36
NORTH CENTRAL HARDWOOD FORESTS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.36
NORTHERN LAKES AND FORESTS, NORTHWESTERN GLACIATED PLAINS	0.35
CENTRAL OLKAHOMA/TEXAS PLAINS, NORTHERN GLACIATED PLAINS	0.35
WESTERN CORN BELT PLAINS, NORTHERN LAKES AND FORESTS	0.34
SOUTHWESTERN TABLELANDS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.34

DRIFTLESS AREA, NORTHWESTERN GLACIATED PLAINS	0.34
WESTERN CORN BELT PLAINS, DRIFTLESS AREA	0.34
CENTRAL OLKAHOMA/TEXAS PLAINS, NORTHERN LAKES AND FORESTS	0.34
WESTERN CORN BELT PLAINS, CENTRAL OLKAHOMA/TEXAS PLAINS	0.33
CENTRAL IRREGULAR PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS	0.33
INTERIOR RIVER VALLEYS AND HILLS, LAKE AGASSIZ PLAIN	0.32
CENTRAL CORN BELT PLAINS, CENTRAL OLKAHOMA/TEXAS PLAINS	0.32
SOUTHWESTERN TABLELANDS, NORTHWESTERN GREAT PLAINS	0.32
CENTRAL GREAT PLAINS, SOUTHWESTERN TABLELANDS	0.32
NORTHERN LAKES AND FORESTS, NORTHERN GLACIATED PLAINS	0.31
CENTRAL OLKAHOMA/TEXAS PLAINS, LAKE AGASSIZ PLAIN	0.30
SOUTHWESTERN TABLELANDS, NORTHERN GLACIATED PLAINS	0.27
INTERIOR RIVER VALLEYS AND HILLS, NORTHERN GLACIATED PLAINS	0.26
CENTRAL IRREGULAR PLAINS, SOUTHWESTERN TABLELANDS	0.26
SOUTHWESTERN TABLELANDS, LAKE AGASSIZ PLAIN	0.22
CENTRAL IRREGULAR PLAINS, NORTHERN GLACIATED PLAINS	0.20
CENTRAL IRREGULAR PLAINS, LAKE AGASSIZ PLAIN	0.18
WESTERN CORN BELT PLAINS, NORTHWESTERN GREAT PLAINS	0.17

Table 4. Mean, 95% confidence intervals, minimum, maximum, and candidate threshold values (CTV) based on percentiles for nutrient concentration in Plains/Upper Midwest streams, WSA aggregated plains ecoregions (see Table 1), and Corn Belt Plains (CBP) groups, reference and non-reference sites, and sites in Illinois or Indiana and sites not in Illinois or Indiana. Percentiles are 25th (and 10th for UMW) except "reference" for which percentiles are 75th. WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

Citan			Total	N (ug/L)			Total P (ug/L)							
Sites	n —	Mean	95%CI	Min	Max	CTV	Mean	95%CI	Min	Max	CTV			
All	327	2955	2411-3498	109	43650	616	216	163-269	2	5418	34			
TPL	130	5252	4079-6551	238	43650	1046	240	164-317	15	4175	65			
UMW	55	1778	1018-2536	160	15650	508 (311)	85	51-120	3	618	17 (9)			
NPL	94	1345	839-1850	114	18775	569	283	139-426	2	5418	37			
SPL	48	1236	808-1664	109	6981	414	169	74-265	3	2034	16			
СВР	61	8539	6409-10669	376	43650	2292	234	91-378	15	4175	61			
Not CBP	266	1674	1374-1974	109	18775	556	212	155-269	2	5418	29			
Reference	50	840	574-1105	109	4454	900	48	34-62	2	181	69			
Not Reference	277	3337	2707-3966	131	43650	3295	247	184-309	4	5418	232			
IN and IL	28	5149	2593-7705	376	33350	889	216	86-347	29	1632	58			
Not IN or IL	299	2749	2206-3293	109	43650	583	216	158-273	2	5418	32			

Table 5. Mean nutrient concentration, percent of watershed area in row crops, and percent canopy openness by level 3 ecoregion.

Level 3 ecoregion	n	Mean total N (ug/L)	Mean total P (ug/L)	Row crops (%)	Canopy openness (%)
WESTERN CORN BELT PLAINS	42	9652	274	79	35
CENTRAL CORN BELT PLAINS	10	9612	133	67	26
SOUTHEASTERN WISCONSIN TILL PLAINS	5	6706	54	56	49
DRIFTLESS AREA	11	5773	166	43	19
HURON/ERIE LAKE PLAINS	3	3803	157	63	23
NORTHWESTERN GLACIATED PLAINS	13	2530	452	21	51
NORTHERN GLACIATED PLAINS	16	2504	365	46	48
EASTERN CORN BELT PLAINS	9	2153	162	74	16
INTERIOR RIVER VALLEYS AND HILLS	17	2080	237	50	27
CENTRAL GREAT PLAINS	11	2004	438	50	38
LAKE AGASSIZ PLAIN	13	1805	307	66	42
SOUTHWESTERN TABLELANDS	18	1231	75	8	41
CENTRAL IRREGULAR PLAINS	13	1216	148	13	16
TEXAS BLACKLAND PRAIRIES	2	1181	163	2	13
S. MICHIGAN/N. INDIANA DRIFT PLAINS	9	1156	114	40	12
NORTHWESTERN GREAT PLAINS	81	1155	255	17	46
FLINT HILLS	2	1102	224	4	18
NORTH CENTRAL HARDWOOD FORESTS	8	958	89	24	24
WESTERN HIGH PLAINS	5	893	28	12	30
CENTRAL OLKAHOMA/TEXAS PLAINS	9	814	154	10	32
NORTHERN MINNESOTA WETLANDS	1	638	45	1	19
NEBRASKA SAND HILLS	1	613	86	1	78
NORTHERN LAKES AND FORESTS	26	599	42	5	27
EDWARDS PLATEAU	2	190	5	0	49

Table 6. Mean values for human disturbance variables. See Appendix 10 for explanation of variables. WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

Sites (n)	Row crops (%)	Pasture/Hay (%)	Developed (%)	Forest (%)	Wetlands (%)	Population density (no./km²)	Road density (km/km²)	Riparian Disturbance (Index score)	Riparian canopy openness (%)
All (327)	34.8	8.8	5.5	15.9	3.4	19.4	1.4	1.3	35.5
TPL (130)	59.6	13.1	7.5	8.5	1.9	25.2	1.8	1.5	32.3
UMW (55) NPL (94)	20.8 17.5	10.1 5.4	5.3 2.1	44.8 7.5	12.1 1.6	21.8 1.5	1.3 0.9	0.5 1.4	22.5 46.5
SPL (48)	17.5	2.0	6.6	19.4	0.9	33.4	1.4	1.4	37.5
CBP (61)	76.5	4.6	9.2	4.6	0.6	35.5	2.2	1.6	30.7
Not CBP (266)	25.2	9.7	4.6	18.5	4.0	15.6	1.2	1.2	36.6
Reference (50)	20.3	9.0	3.5	30.8	3.4	6.5	1.1	0.9	27.0
Not Reference (277)	37.4	8.7	5.8	13.2	3.4	21.9	1.5	1.3	37.0
IN and IL (28)	66.6	6.3	13.1	13.0	0.1	70.6	1.9	1.7	24.3
Not IN or IL (299)	31.8	9.0	4.7	16.2	3.7	14.4	1.3	1.2	36.5

Table 7. Significant linear regression models for predicting nutrient concentrations (CTVs) at background human disturbance (backtransformed *y*-intercept). Disturbance data are from NLCD 2001 (% land cover), and WSA site data. *Df* is degrees of freedom; *Cp* is Mallows statistic which was used to select the most adequate significant model; prediction is the back transformed *y*-intercept (the nutrient concentration in ug/L at a site with no disturbance). WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

	Y (log-	y-intercept _			Re	egression coef	ficients			Frror			CTV
Sites	transform ed)	(SE)	Row crops %	Pasture/ hay %	Develop ment %	Population density No/km ²	Road density Km/km ²	Riparian disturbance Score	Canopy openness % 0.0022 307 0.54 5.34 77 0.18 5.76 42 0.48 4.30 0.0027 123 0.44 4.78 52 0.61 2.86 57 0.22 3.53 24 0.34 4.16 1 0.0041 306 0.17 6.19 75 0.09 na 42 0.30 3.50	(ug/L)			
All	Total N	2.49 (0.05)	0.0104	0.0038			0.1152		0.0022	307	0.54	5.34	306
NPL	Total N	2.72 (0.13)	0.0069	0.0017		0.0082	0.0433	0.0007		77	0.18	5.76	526
SPL	Total N	2.57 (0.07)	0.0120	0.0196		0.0006				42	0.48	4.30	370
TPL	Total N	2.46 (0.11)	0.0077			-0.0009	0.2532		0.0027	123	0.44	4.78	288
UMW	Total N	2.60 (0.06)	0.0115	0.0128						52	0.61	2.86	397
CBP	Total N	2.52 (0.31)	0.0106				0.1629	0.0216		57	0.22	3.53	333
IN + IL	Total N	2.30 (0.34)	0.0134		0.0158			0.0303		24	0.34	4.16	196
All	Total P	1.43 (0.08)	0.0058	0.0068		0.0002		0.0661	0.0041	306	0.17	6.19	26
NPL	Total P	1.87 (0.29)			Reg	gression not sig	gnificant			75	0.09	na	73
SPL	Total P	1.37 (0.12)	0.0142	0.0313		0.0012				42	0.30	3.50	23
TPL	Total P	1.85 (0.18)			Reg	gression not sig	gnificant			120	0.07	na	70
UMW	Total P	1.31 (0.09)	0.0079	0.0160				-0.0091		51	0.35	3.52	19
CBP	Total P	1.97 (0.50)			Reg	gression not sig	gnificant			53	0.09	na	92
IN + IL	Total P	1.55 (0.24)				-0.0034	0.3898			25	0.20	2.96	34

Table 8. CTVs for total N based on interpretation of piecewise regression models. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 6. Metric values decrease with increasing nutrients unless indicated by "(+)". Back-transformed data given in Appendix 11.

		Piecewi	se model i	nterpretation	based on	raw metrics			Piecewise 1	nodel inter	pretation bas	sed on reg	ression resid	duals
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)
OLLEPTAX (+)	0.15		4.08	4.64	4.36	4.08	12022	0.12		4.08	4.64	4.36	4.08	12022
TL07RICH	0.16	2.04	3.11		2.58	2.58	379	0.18		2.52	2.87	2.70	2.52	330
HBI (+)	0.14		2.9	2.92	2.91	2.91	812	0.09		2.39	3.01	2.70	2.39	244
PLECRICH	0.22		2.32	2.61	2.47	2.47	294	0.14	2.04		2.68	2.36	2.36	228
ODONRICH	0.09		2.71	2.81	2.76	2.71	512	0.10		2.59	4.64	3.62	2.59	388
CLMBRICH	0.08		2.52	4.64	3.58	2.52	330	0.12		3.39	4.64	4.02		
TL89PTAX (+)	0.08	2.04		3.13	2.59	2.59	388	0.03	2.04		3.14	2.59	2.59	388
TL01RICH	0.25		2.3	2.63	2.47	2.47	294	0.21		2.28	2.87	2.58	2.58	379
SHRDRICH	0.09	2.04	2.88		2.46	2.46	287	0.09	2.04		2.93	2.49	2.49	308
HABT_PT	0.07		2.44	2.44	2.44	2.44	274	0.04	2.04		3.33	2.69	2.69	489
TL67RICH	0.06		2.5	3.08	2.79	2.50	315	0.08	2.04		3.31	2.68	2.68	478
SCRPRICH	0.04	2.04	3.11		2.58	2.58	379	0.04	2.04		4.64	3.34		
TOLR_PT	0.09	2.04	3.05		2.55	2.55	354	0.05	2.04		3.01	2.53	2.53	338
TL03PIND	0.11		2.32	3.01	2.67	2.67	467	0.09		2.32	2.97	2.65	2.65	446
MMI_WSABEST	0.14	2.04	3.14		2.59	2.59	388	0.07		2.17	2.20	2.19		
CHIRRICH	0.07	2.04	3.63		2.84	2.84	691	0.11	2.04		3.99	3.02	3.02	1046
PREDRICH	0.17		2.16	2.23	2.20			0.16		2.09	2.28	2.19	2.28	190
HPRIME	0.08	2.04		3.28	2.66	2.66	456	0.14	2.04		3.28	2.66	2.66	456
SPRLRICH	0.12	2.04		3.07	2.56	2.56	362	0.16	2.04		2.99	2.52	2.52	330
INTLRICH	0.21		2.37	2.63	2.50	2.50	315	0.21		2.35	2.87	2.61	2.61	406
FEED_PT	0.07		2.53	2.62	2.58	2.58	379	0.04		3.57	3.59	3.58	3.57	3714
COGARICH	0.09	2.04		3.12	2.58	2.58	379	0.12	2.04		4.00	3.02	3.02	1046
EPT_RICH	0.15		2.37	2.61	2.49	2.49	308	0.13		2.36	2.75	2.56	2.56	362
EPHE_PT	0.12		3.04	3.08	3.06	3.04	1095	0.05	2.04		4.64	3.34		
Median	0.10						379	0.11						388

Table 9. CTVs for total P based on interpretation of piecewise regression models. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 6. Metric values decrease with increasing nutrients unless indicated by "(+)". Back-transformed data given in Appendix 11.

	P	iecewise 1	model int	erpretation	based o	on raw me	trics	Piecewise model interpretation based on regression residuals						
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)
OLLEPTAX (+)	0.05	0.48		3.73	2.11			0.06	0.48		3.73	2.11		
TL07RICH	0.22		1.55	1.56	1.56	1.55	34	0.17		1.63	1.63	1.63	1.63	42
HBI (+)	0.09		2.75	3.07	2.91	2.75	561	0.05	0.48		3.73	2.11		
PLECRICH	0.10	0.48		1.72	1.10	1.10	12	0.10		1.28	1.49	1.28	1.39	24
ODONRICH	0.05	0.48		2.01	1.25	1.25	17	0.06		1.78	1.78	1.78	1.78	59
CLMBRICH	0.09		1.50	3.73	2.62	1.50	31	0.10		1.73	1.79	1.76	1.76	57
TL89PTAX (+)	0.08	0.48		3.73	2.11			0.05	0.48		2.66	1.57	1.57	36
TL01RICH	0.18		1.34	1.34	1.34	1.34	21	0.16	0.48		2.02	1.25	1.25	17
SHRDRICH	0.11	0.48		1.98	1.23	1.23	16	0.09	0.48		2.00	1.24	1.24	16
HABT_PT	0.10	0.48		1.15	0.82	0.82	6	0.05	0.48		1.15	0.82	0.82	6
TL67RICH	0.11		1.48	2.49	1.99	1.48	29	0.09		1.54	1.79	1.67	1.54	34
SCRPRICH	0.10	0.48		3.73	2.11			0.07	0.48	2.18		1.33	1.33	20
TOLR_PT	0.07	0.48		3.73	2.11			0.05		0.79	0.90	0.85	0.9	7
TL03PIND	0.07		0.87	1.04	0.96	0.96	8	0.06		0.86	0.88	0.87		
MMI_WSABEST	0.11	0.48		3.73	2.11			0.06	0.48		3.73	2.11		
CHIRRICH	0.11		1.45	3.73	1.89	1.45	27	0.07		1.45	3.73	2.59	1.45	27
PREDRICH	0.11		1.27	3.73	2.50	1.27	18	0.06	0.48		1.96	1.22	1.22	16
HPRIME	0.14		2.57	3.73	3.15	2.57	371	0.10		2.78	3.73	3.26	2.78	602
SPRLRICH	0.15		1.22	4.64	2.93	1.22	16	0.12	0.48		1.98	1.23	1.23	16
INTLRICH	0.19	0.48		1.75	1.12	1.12	12	0.17	0.48		2.01	1.25	1.25	17
FEED_PT	0.08		1.61	1.62	1.62	1.61	40	0.06		1.61	1.62	1.62	1.61	40
COGARICH	0.16		1.21	3.73	2.47	1.21	15	0.12		1.00	3.73	2.37	1	9
EPT_RICH	0.13		1.00	1.10	1.05	1.05	10	0.09	0.48		1.94	1.21	1.21	15
EPHE_PT	0.04	0.48		1.69	1.09	1.09	11	0.02	0.48		3.73	2.11		
Median	0.11						17	0.07						20

Table 10. CTVs for total N based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are relationships without reliable breakpoints. Data plotted in Appendix 7. Metric values decrease with increasing nutrients unless indicated by "(+)".Back-transformed data given in Appendix 11.

.Buck trunsform		ewise mo	del inter		based o	on raw met	trics for	Piecewise model interpretation base on raw metrics for streams with coarse substrates (>1mm)							
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	
OLLEPTAX (+)	0.13		4.08	4.33	4.21	4.08	12022	0.15	2.06			2.06			
TL07RICH	0.16		2.52	2.62	2.57	2.57	371	0.18	2.06	3.23		2.65	2.65	446	
HBI (+)	0.11		2.90	3.01	2.96	2.96	911	0.14		2.96	4.64	3.80	2.96	911	
PLECRICH	0.22		2.52	2.53	2.53	2.53	338	0.22		2.31	2.50	2.41	2.41	256	
ODONRICH	0.12		2.73	2.74	2.74	2.74	549	0.12		2.05	4.64	3.35			
CLMBRICH	0.09		2.75	2.75	2.75	2.75	561	0.11		3.45	4.64	4.05	3.45	2817	
TL89PTAX (+)	0.11		2.90	3.01	2.96	2.96	911	0.07	2.06	2.97		2.52	2.52	330	
TL01RICH	0.20	2.04	2.88		2.46	2.46	287	0.27		2.31	2.48	2.40	2.40	250	
SHRDRICH	0.07		2.54	2.56	2.55	2.55	354	0.21		2.24	3.09	2.67	2.67	467	
HABT_PT	0.05		3.01	3.02	3.02	3.02	1046	0.18	2.06	3.22		2.64	2.64	436	
TL67RICH	0.08		2.54	2.73	2.64	2.64	436	0.09		2.77	3.01	2.89	2.77	588	
SCRPRICH	0.02		2.37	4.33	3.35			0.07	2.06	3.45		2.76	2.76	574	
TOLR_PT	0.06	2.04	2.98		2.51	2.51	323	0.10	2.06	2.86		2.46	2.46	287	
TL03PIND	0.08		2.28	3.12	2.70	2.70	500	0.17	2.06	3.23		2.65	2.65	446	
MMI_WSABEST	0.11	2.04	3.31		2.68	2.68	478	0.13		2.32	2.69	2.51	2.51	323	
CHIRRICH	0.09	2.04	3.01		2.53	2.53	338	0.06	2.06	3.93		3.00			
PREDRICH	0.12	2.04	2.75		2.40			0.07	2.06			2.06			
HPRIME	0.06	2.04	3.13		2.59	2.59	388	0.07		2.59	3.12	2.86	2.59	388	
SPRLRICH	0.09	2.04	2.96		2.50	2.50	315	0.12		2.29	3.02	2.66	2.66	456	
INTLRICH	0.16		2.58	2.58	2.58	2.58	379	0.19	2.06	3.22		2.64	2.64	436	
FEED_PT	0.06		2.39	2.75	2.57	2.57	371	0.08	2.06	2.12		2.09			
COGARICH	0.10	2.04	3.00		2.52	2.52	330	0.07		2.59	3.13	2.86	2.59	388	
EPT_RICH	0.11		2.43	2.63	2.53	2.53	338	0.13	2.06	3.28		2.67	2.67	467	
EPHE_PT	0.10		2.90	3.30	3.10	3.10	1258	0.09	2.06	2.11		2.09			
Median	0.10						384	0.12						441	

Table 11. CTVs for total P based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 7. Metric values decrease with increasing nutrients unless indicated by "(+)".Back-transformed data given in Appendix 11.

Maria	Piec	Piecewise model interpretation based on raw metrics for streams with fine substrates (<1 mm)								Piecewise model interpretation base on raw metrics for streams with coarse substrates (≥1mm)					
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	
OLLEPTAX (+)	0.13	0.48	3.61		2.05			0.16		1.86	2.74	2.30	1.86	71	
TL07RICH	0.15		1.52	1.67	1.60	1.60	39	0.35		1.51	2.74	2.13	1.51	31	
HBI (+)	0.07		2.75	3.06	2.91	2.75	561	0.17	0.61	2.15		1.38	1.38	23	
PLECRICH	0.07	0.48	1.42		0.95	0.95	8	0.31		1.30	1.31	1.31	1.31	19	
ODONRICH	0.08	0.48	2.63		1.56	1.56	35	0.06	0.61	2.01		1.31	1.31	19	
CLMBRICH	0.10		1.32	3.73	2.53	1.32	20	0.09	0.61	0.87		0.74			
TL89PTAX (+)	0.07		1.08	3.35	2.22			0.15	0.61	2.28		1.45	1.45	27	
TL01RICH	0.20		0.93	1.01	0.97	0.97	8	0.20		1.06	2.74	1.90	1.06	10	
SHRDRICH	0.08	0.48	1.91		1.20	1.20	15	0.20	0.61	2.37		1.49	1.49	30	
HABT_PT	0.06	0.48	3.19		1.84			0.15	0.61	0.64		0.63			
TL67RICH	0.11		1.67	1.72	1.70	1.70	49	0.25		1.33	1.42	1.38	1.42	25	
SCRPRICH	0.07		1.31	3.73	2.52	1.31	19	0.13		0.87	0.89	0.88			
TOLR_PT	0.04		1.21	3.73	2.47	1.21	15	0.17		2.51	2.74	2.63			
TL03PIND	0.03		2.75	3.73	3.24	2.75	561	0.11		1.03	2.74	1.89	1.03	10	
MMI_WSABEST	0.07		2.31	3.73	3.02	2.31	203	0.23	0.61	2.26		1.44	1.44	27	
CHIRRICH	0.11		1.05	1.13	1.09			0.34		1.76	2.74	2.25	1.76	57	
PREDRICH	0.06		2.88	3.73	3.31			0.23		1.04	2.74	1.89	1.04	10	
HPRIME	0.12		2.59	3.73	3.16	2.59	388	0.19		1.51	2.74	2.13	1.51	31	
SPRLRICH	0.12		1.04	1.12	1.08			0.35		1.56	2.74	2.15	1.56	35	
INTLRICH	0.16		0.97	0.97	0.97			0.29		1.00	2.74	1.87	1.00	9	
FEED_PT	0.06	0.48		3.73	2.11			0.10	0.61	2.26		1.44	1.44	27	
COGARICH	0.14		1.21	3.73	1.81	1.21	15	0.25		1.56	2.74	2.15	1.56	35	
EPT_RICH	0.09		0.98	0.98	0.98			0.22		1.06	1.66	1.36	1.36	22	
EPHE_PT	0.02	0.48	0.00	3.73	1.40			0.06	0.61		2.74	1.68			
Median	0.08						28	0.20						27	

Table 12. Candidate threshold values (CTVs) for total N based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 8. Asterisk indicates a reference value (47.6) from Herlihy and Sifneos (2008). Metric values decrease with increasing nutrients unless indicated by "(+)".

Mark	25 th percentile		75 th percentile		Med	r^2	CTV.	CTV	50% pre	50% prediction	
Metric	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r	CTV	(ug/L)	limits (
OLLEPTAX (+)	2.9	2.5	7.0	4.0	4.3	3.2	0.12	2.38	238	2208	25
TL07RICH	16.0	23.0	28.0	33.0	22.0	27.0	0.12	2.47	297	32	2688
HBI (+)	5.2	4.6	6.6	5.7	5.8	5.3	0.06	2.23	167	4553	5
PLECRICH	0.0	0.0	0.0	2.0	0.0	0.0	0.09	3.75	5570	425	73941
ODONRICH	0.0	0.0	1.0	1.0	1.0	1.0	0.07	2.78	606	31	11584
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.07	3.20	1568	85	28854
TL89PTAX (+)	13.8	11.1	25.0	20.5	20.0	15.1	0.05	1.94	85	3384	1
TL01RICH	0.0	0.0	1.0	3.0	0.0	1.0	0.14	3.04	1086	141	8357
SHRDRICH	2.0	3.0	5.0	6.0	4.0	4.0	0.05	3.00	1005	29	33248
HABT_PT	1.7	3.7	5.3	6.7	3.6	5.1	0.04	1.89	77	1	3420
TL67RICH	5.0	7.0	10.0	10.0	8.0	8.0	0.06	3.00	1001	35	27903
SCRPRICH	2.0	3.0	5.0	6.0	3.0	4.0	0.03	2.54	347	2	49102
TOLR_PT	1.3	3.9	5.7	8.5	3.4	5.9	0.02	0.46	2	-1	1028
TL03PIND	2.7	9.7	23.3	33.3	9.0	20.3	0.02	2.26	180	0	36715
MMI_WSABEST	21.8	35.7	45.3	65.0	33.4	50.5	0.10	1.93	84	6	1004
MMI_WSABEST*						47.6	0.10	2.19	153	12	1820
CHIRRICH	9.0	10.0	16.0	15.0	12.0	13.0	0.04	2.88	750	11	48146
PREDRICH	5.0	6.0	11.0	13.0	8.0	10.0	0.15	2.58	384	56	2591
HPRIME	1.9	2.2	2.7	2.8	2.4	2.6	0.04	1.97	93	1	5072
SPRLRICH	6.0	9.0	12.0	14.0	9.0	10.0	0.08	2.94	874	60	12662
INTLRICH	1.0	2.0	5.0	8.0	3.0	4.0	0.09	3.02	1037	78	13706
FEED_PT	1.3	2.5	5.0	7.1	2.9	5.4	0.06	1.57	36	0	998
COGARICH	10.0	12.0	17.0	18.0	14.0	15.0	0.05	2.66	454	15	13542
EPT_RICH	2.0	4.0	7.0	11.0	4.0	7.0	0.08	2.52	332	21	4993
EPHE_PT	1.0	1.4	4.3	7.1	2.0	3.9	0.09	2.63	429	34	5306
Median							0.07		347	31	5306

Table 13. Candidate threshold values (CTVs) for total P based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 8. Asterisk indicates a reference value (47.6) from Herlihy and Sifneos (2008). Metric values decrease with increasing nutrients unless indicated by "(+)".

Metric	25 th pero	centile	75 th percentile		Med		OTT I	CTV	50% pred	diction		
·	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r^2	CTV	(ug/L)		limits (ug/L)	
OLLEPTAX (+)	2.9	2.5	7.0	4.0	4.3	3.2	0.04	0.58	3	251	-1	
TL07RICH	16.0	23.0	28.0	33.0	22.0	27.0	0.20	1.38	23	3	142	
HBI (+)	5.2	4.6	6.6	5.7	5.8	5.3	0.06	1.00	9	309	-1	
PLECRICH	0.0	0.0	0.0	2.0	0.0	0.0	0.08	2.64	436	22	8202	
ODONRICH	0.0	0.0	1.0	1.0	1.0	1.0	0.05	1.47	29	0	1674	
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.09	2.01	100	5	1851	
TL89PTAX (+)	13.8	11.1	25.0	20.5	20.0	15.1	0.07	0.86	6	191	-1	
TL01RICH	0.0	0.0	1.0	3.0	0.0	1.0	0.14	1.84	68	7	629	
SHRDRICH	2.0	3.0	5.0	6.0	4.0	4.0	0.10	1.84	68	4	1005	
HABT_PT	1.7	3.7	5.3	6.7	3.6	5.1	0.06	0.81	5	-1	195	
TL67RICH	5.0	7.0	10.0	10.0	8.0	8.0	0.09	1.83	66	3	1170	
SCRPRICH	2.0	3.0	5.0	6.0	3.0	4.0	0.09	1.59	38	1	649	
TOLR_PT	1.3	3.9	5.7	8.5	3.4	5.9	0.07	0.43	2	-1	67	
TL03PIND	2.7	9.7	23.3	33.3	9.0	20.3	0.06	1.33	21	0	792	
MMI_WSABEST	21.8	35.7	45.3	65.0	33.4	50.5	0.11	0.72	4	-1	62	
MMI_WSABEST*						47.6	0.11	0.98	9	0	114	
CHIRRICH	9.0	10.0	16.0	15.0	12.0	13.0	0.09	1.76	57	3	934	
PREDRICH	5.0	6.0	11.0	13.0	8.0	10.0	0.11	1.22	16	0	221	
HPRIME	1.9	2.2	2.7	2.8	2.4	2.6	0.13	1.22	16	1	173	
SPRLRICH	6.0	9.0	12.0	14.0	9.0	10.0	0.15	1.78	60	6	510	
INTLRICH	1.0	2.0	5.0	8.0	3.0	4.0	0.18	1.85	70	9	488	
FEED_PT	1.3	2.5	5.0	7.1	2.9	5.4	0.07	0.43	2	-1	63	
COGARICH	10.0	12.0	17.0	18.0	14.0	15.0	0.15	1.62	41	4	361	
EPT_RICH	2.0	4.0	7.0	11.0	4.0	7.0	0.11	1.35	21	1	298	
EPHE_PT	1.0	1.4	4.3	7.1	2.0	3.9	0.04	1.11	12	-1	1011	
Median							0.09		21	3	361	

Table 14. Candidate threshold values (CTVs) for total N for streams with fine substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

Metric	25 th pe	rcentile	75 th per	rcentile	Med	dian			CTV
	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r^2	CTV	(ug/L)
OLLEPTAX (+)	2.9	2.4	7.1	5.6	4.3	3.7	0.10	2.56	365
TL07RICH	14.0	22.0	27.0	35.0	21.0	27.0	0.10	2.22	164
HBI (+)	5.3	4.8	6.7	5.9	5.9	5.5	0.03	2.05	112
PLECRICH	0.0	0.0	0.0	2.0	0.0	0.0	0.05	3.87	7369
ODONRICH	0.0	1.0	1.0	2.0	1.0	1.0	0.09	2.99	968
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.09	3.32	2065
TL89PTAX (+)	13.8	12.5	27.3	20.7	20.0	16.7	0.04	2.13	135
TL01RICH	0.0	1.0	1.0	3.0	0.0	1.0	0.12	2.87	746
SHRDRICH	2.0	3.0	5.0	6.0	4.0	6.0	0.03	-0.04	0
HABT_PT	1.2	3.5	5.0	5.8	3.5	4.7	0.03	1.71	50
TL67RICH	5.0	7.0	10.0	11.0	7.0	8.0	0.07	3.00	1003
SCRPRICH	2.0	1.0	4.0	5.0	3.0	4.0	0.01	1.18	14
TOLR_PT	1.3	3.8	5.6	7.9	3.3	4.6	0.01	0.68	4
TL03PIND	2.0	9.7	19.7	37.7	7.0	14.7	0.01	2.72	524
MMI_WSABEST	20.3	31.2	41.4	54.6	31.5	43.9	0.06	1.82	66
MMI_WSABEST*						47.6	0.06	1.35	21
CHIRRICH	8.0	11.0	15.0	20.0	12.0	15.0	0.04	1.89	76
PREDRICH	5.0	6.0	10.0	12.0	7.0	10.0	0.12	2.34	219
HPRIME	1.8	2.0	2.7	2.9	2.4	2.6	0.03	1.62	41
SPRLRICH	6.0	9.0	11.0	15.0	9.0	10.0	0.07	2.84	698
INTLRICH	1.0	2.0	4.0	7.0	2.0	3.0	0.05	3.39	2459
FEED_PT	1.3	0.0	4.3	7.1	2.5	5.7	0.03	0.15	0
COGARICH	10.0	14.0	16.0	21.0	13.0	17.0	0.06	1.83	67
EPT_RICH	2.0	3.0	6.0	11.0	4.0	5.0	0.05	3.03	1079
EPHE_PT	1.0	1.4	4.0	6.3	1.4	2.0	0.07	3.66	4588
Median							0.05		164

Table 15. Candidate threshold values (CTVs) for total N for streams with coarse substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

Metric	25 th pe	rcentile	75 th pe	rcentile	Me	dian		CTV	CTV	
	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r^2	CTV	(ug/L)	
OLLEPTAX (+)	3.1	2.5	6.5	3.7	4.3	3.1	0.15	2.33	213	
TL07RICH	21.0	23.0	30.0	33.0	26.0	27.0	0.08	2.84	695	
HBI (+)	5.0	4.4	6.0	5.6	5.5	4.9	0.07	2.11	128	
PLECRICH	0.0	0.0	1.0	2.0	0.0	0.0	0.11	3.73	5323	
ODONRICH	0.0	0.0	1.0	1.0	1.0	1.0	0.07	2.37	236	
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.08	2.86	727	
TL89PTAX (+)	12.3	10.0	23.4	20.5	18.8	13.8	0.04	1.76	57	
TL01RICH	0.0	0.0	1.0	3.0	0.0	1.0	0.15	3.11	1291	
SHRDRICH	2.0	3.0	5.0	6.0	4.0	4.0	0.12	2.96	906	
HABT_PT	2.6	4.2	6.2	7.1	4.5	5.9	0.02	1.24	17	
TL67RICH	6.5	6.0	10.0	10.0	8.0	8.0	0.02	3.29	1946	
SCRPRICH	3.0	3.0	6.0	6.0	4.0	5.0	0.01	1.79	60	
TOLR_PT	1.5	3.9	5.8	8.6	3.7	6.9	0.03	0.66	4	
TL03PIND	7.3	9.7	30.0	34.3	15.5	23.3	0.17	2.70	500	
MMI_WSABEST	29.5	39.7	60.0	68.9	42.3	52.4	0.08	2.40	251	
MMI_WSABEST*	10.0					47.6	0.08	2.82	653	
CHIRRICH	6.0	10.0	16.0	14.0	14.0	12.0	0.01	4.14	13701	
PREDRICH	2.2	8.0	12.0	14.0	10.0	10.0	0.14	2.91	811	
HPRIME	8.0	2.2	2.9	2.8	2.6	2.6	0.02	2.12	130	
SPRLRICH	2.0	9.0	13.0	14.0	10.0	10.0	0.09	3.16	1428	
INTLRICH	2.0	2.0	6.0	9.0	4.0	4.0	0.09	3.40	2511	
FEED_PT	11.0	2.9	6.3	7.1	4.0	5.0	0.06	2.46	286	
COGARICH	4.0	12.0	18.0	16.0	15.0	14.0	0.02	3.45	2822	
EPT_RICH	1.7	5.0	10.0	12.0	7.0	8.0	0.06	2.77	593	
EPHE_PT	1.0	2.0	5.7	7.1	3.0	4.3	0.07	2.80	636	
Median							0.07		636	

Table 16. Candidate threshold values (CTVs) for total P for streams with fine substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

Metric	25 th pe	rcentile	75 th per	75 th percentile		dian		CTV	CTV	
	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r2	CTV	(ug/L)	
OLLEPTAX (+)	2.9	2.4	7.1	5.6	4.3	3.7	0.02	0.33	1	
TL07RICH	14.0	22.0	27.0	35.0	21.0	27.0	0.14	1.05	10	
HBI (+)	5.3	4.8	6.7	5.9	5.9	5.5	0.03	0.80	5	
PLECRICH	0.0	0.0	0.0	2.0	0.0	0.0	0.04	2.82	658	
ODONRICH	0.0	1.0	1.0	2.0	1.0	1.0	0.06	1.73	53	
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.10	2.13	133	
TL89PTAX (+)	13.8	12.5	27.3	20.7	20.0	16.7	0.04	0.89	7	
TL01RICH	0.0	1.0	1.0	3.0	0.0	1.0	0.12	1.64	42	
SHRDRICH	2.0	3.0	5.0	6.0	4.0	6.0	0.08	-0.29	0	
HABT_PT	1.2	3.5	5.0	5.8	3.5	4.7	0.02	-0.06	0	
TL67RICH	5.0	7.0	10.0	11.0	7.0	8.0	0.08	1.79	61	
SCRPRICH	2.0	1.0	4.0	5.0	3.0	4.0	0.06	1.04	10	
TOLR_PT	1.3	3.8	5.6	7.9	3.3	4.6	0.03	0.87	6	
TL03PIND	2.0	9.7	19.7	37.7	7.0	14.7	0.11	2.39	246	
MMI_WSABEST	20.3	31.2	41.4	54.6	31.5	43.9	0.06	0.49	2	
MMI_WSABEST*						47.6	0.06	-0.02	0	
CHIRRICH	8.0	11.0	15.0	20.0	12.0	15.0	0.08	0.92	7	
PREDRICH	5.0	6.0	10.0	12.0	7.0	10.0	0.06	0.60	3	
HPRIME	1.8	2.0	2.7	2.9	2.4	2.6	0.10	0.95	8	
SPRLRICH	6.0	9.0	11.0	15.0	9.0	10.0	0.10	1.67	45	
INTLRICH	1.0	2.0	4.0	7.0	2.0	3.0	0.11	2.15	139	
FEED_PT	1.3	0.0	4.3	7.1	2.5	5.7	0.06	-0.46	-1	
COGARICH	10.0	14.0	16.0	21.0	13.0	17.0	0.13	0.98	9	
EPT_RICH	2.0	3.0	6.0	11.0	4.0	5.0	0.06	1.81	64	
EPHE_PT	1.0	1.4	4.0	6.3	1.4	2.0	0.02	3.17	1461	
Median							0.06		8.6	

Table 17. Candidate threshold values (CTVs) for total P for streams with coarse substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

Maria	25 th percentile		75 th percentile		Me	dian		CTV	CTV	
Metric	Non- reference	Reference	Non- reference	Reference	Non- reference	Reference	r^2	CTV	(ug/L)	
OLLEPTAX (+)	3.1	2.5	6.5	3.7	4.3	3.1	0.15	1.15	13	
TL07RICH	21.0	23.0	30.0	33.0	26.0	27.0	0.30	1.72	51	
HBI (+)	5.0	4.4	6.0	5.6	5.5	4.9	0.10	1.08	11	
PLECRICH	0.0	0.0	1.0	2.0	0.0	0.0	0.14	2.47	294	
ODONRICH	0.0	0.0	1.0	1.0	1.0	1.0	0.03	0.86	6	
CLMBRICH	3.0	3.0	5.0	5.0	4.0	4.0	0.08	1.70	49	
TL89PTAX (+)	12.3	10.0	23.4	20.5	18.8	13.8	0.12	1.08	11	
TL01RICH	0.0	0.0	1.0	3.0	0.0	1.0	0.18	1.93	83	
SHRDRICH	2.0	3.0	5.0	6.0	4.0	4.0	0.18	1.79	60	
HABT_PT	2.6	4.2	6.2	7.1	4.5	5.9	0.14	1.18	14	
TL67RICH	6.5	6.0	10.0	10.0	8.0	8.0	0.09	1.91	81	
SCRPRICH	3.0	3.0	6.0	6.0	4.0	5.0	0.09	1.42	25	
TOLR_PT	1.5	3.9	5.8	8.6	3.7	6.9	0.15	0.82	6	
TL03PIND	7.3	9.7	30.0	34.3	15.5	23.3	0.11	1.67	46	
MMI_WSABEST	29.5	39.7	60.0	68.9	42.3	52.4	0.16	1.38	23	
MMI_WSABEST*	10.0					47.6	0.16	1.68	47	
CHIRRICH	6.0	10.0	16.0	14.0	14.0	12.0	0.11	2.16	144	
PREDRICH	2.2	8.0	12.0	14.0	10.0	10.0	0.18	1.74	54	
HPRIME	8.0	2.2	2.9	2.8	2.6	2.6	0.15	1.48	29	
SPRLRICH	2.0	9.0	13.0	14.0	10.0	10.0	0.30	1.89	76	
INTLRICH	2.0	2.0	6.0	9.0	4.0	4.0	0.27	2.03	107	
FEED_PT	11.0	2.9	6.3	7.1	4.0	5.0	0.05	1.23	16	
COGARICH	4.0	12.0	18.0	16.0	15.0	14.0	0.15	1.97	92	
EPT_RICH	1.7	5.0	10.0	12.0	7.0	8.0	0.15	1.66	45	
EPHE_PT	1.0	2.0	5.7	7.1	3.0	4.3	0.06	1.62	41	
Median							0.15		46	

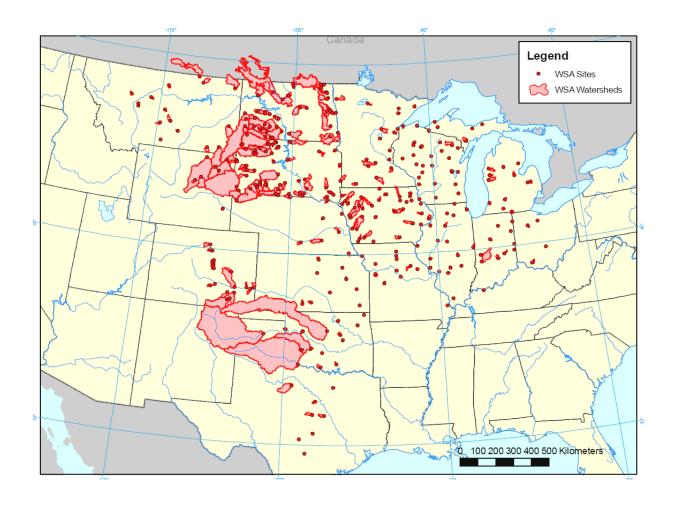


Figure 1. Locations of the 327 Plains/Upper Midwest sites and their watersheds used in this analysis. Portions of watersheds in Canada are ignored.

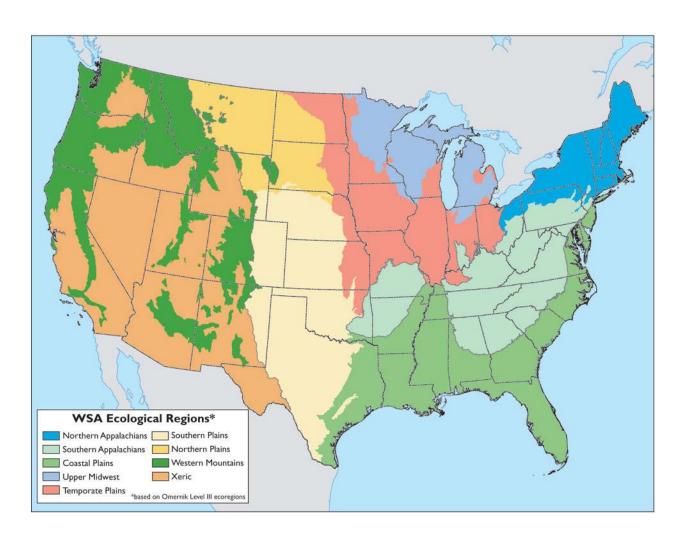
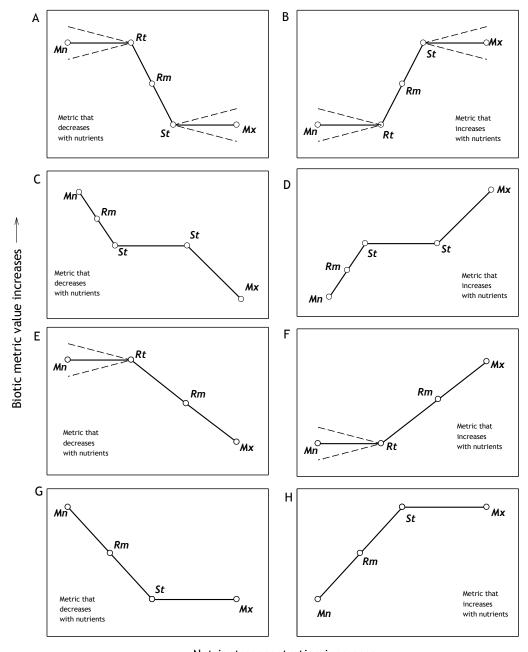
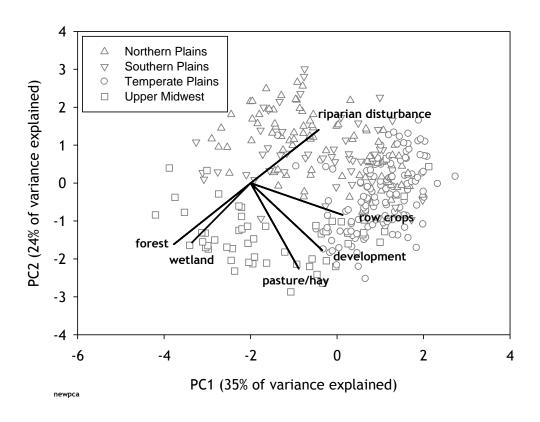


Figure 2. WSA aggregate ecoregions. Aggregate ecoregions are combined level 3 ecoregions. Figure is from http://www.epa.gov/owow/streamsurvey/.



Nutrient concentration increases—>

Fig. 3. Hypothesized expected three- and two-breakpoint metric responses to increasing nutrient concentration. Mn = minimum observed concentration; Rt = response threshold, Rm = response midpoint, St = secondary threshold, Mx = maximum observed concentration. See text for additional details



Variable	PC1	PC2
Riparian disturbance	0.39	0.35
Row crops	0.53	-0.21
Pasture/hay	0.28	-0.56
Development	0.41	-0.44
Forest	-0.44	-0.40
Wetland	-0.34	-0.40

Figure 4. Principal coordinates ordination and eigenvectors for land use and riparian disturbance at Plains/Upper Midwest sites. Symbols indicate WSA aggregate ecoregions.

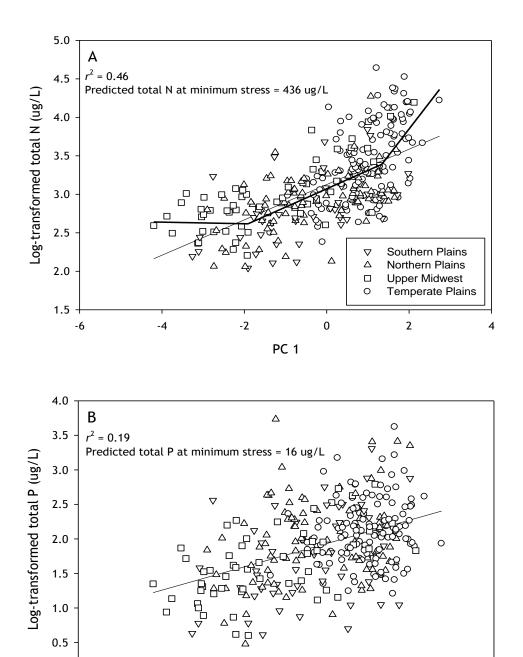


Figure 5. Plot A) Piecewise 3 segment linear regression relationship between a human disturbance gradient (PC 1) and total N (simple linear fit shown for comparison). Plot B) Linear regression relationship between a human disturbance gradient (PC 1) and total P. Variables given in Fig. 4. Symbols indicate WSA aggregate ecoregions.

PC 1

-2

0

2

0.0

-6

-4

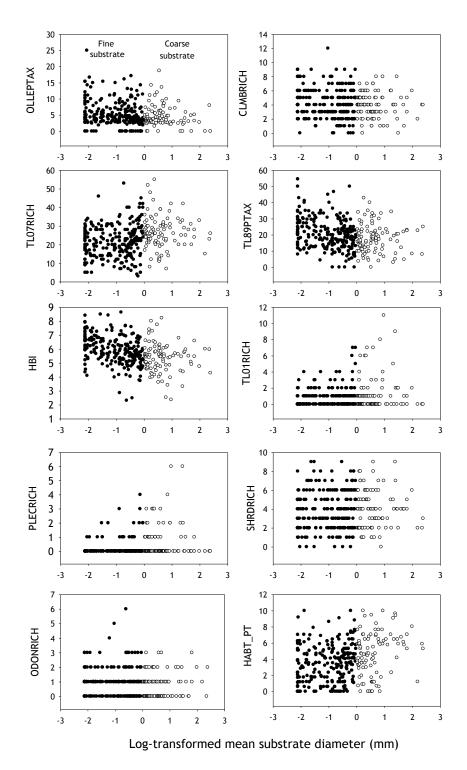


Fig. 6. Relationships between macroinvertebrate metrics and substrate size. Value of zero on x-axis = 1 mm.

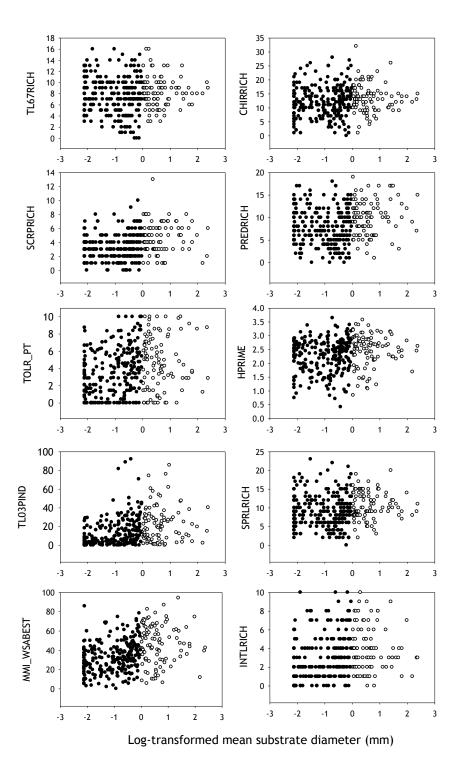
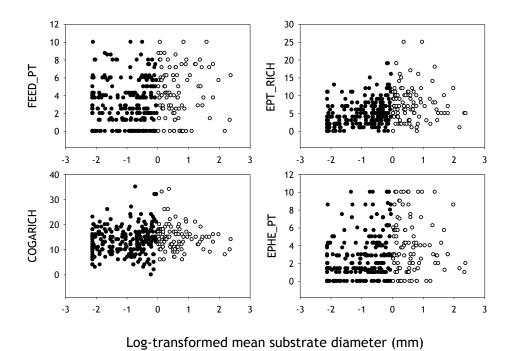


Fig. 6, continued. Relationships between macroinvertebrate metrics and substrate size. Value of zero on x-axis = 1 mm.



on x-axis = 1 mm.

Fig. 6, continued. Relationships between macroinvertebrate metrics and substrate size. Value of zero

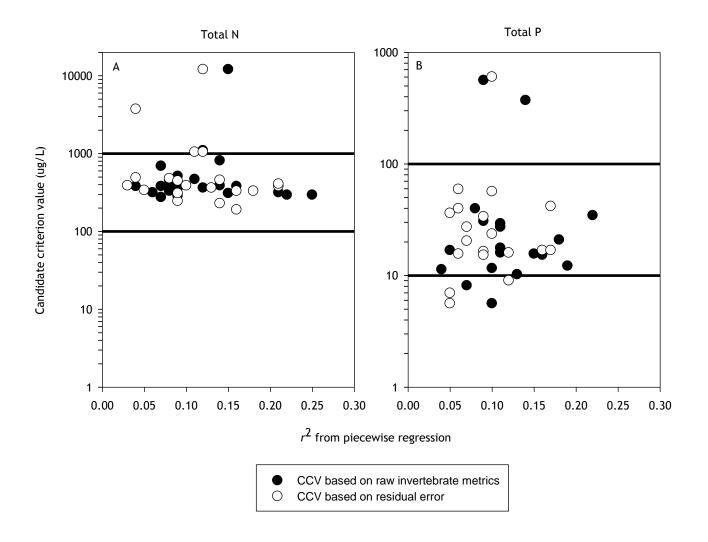


Fig. 7. Candidate threshold values (CTV) plotted against r^2 for the applicable piecewise regression (data from Tables 8 and 9). Horizontal lines at 100 and 1000 ug/L are for reference. CTVs < 1 not shown.

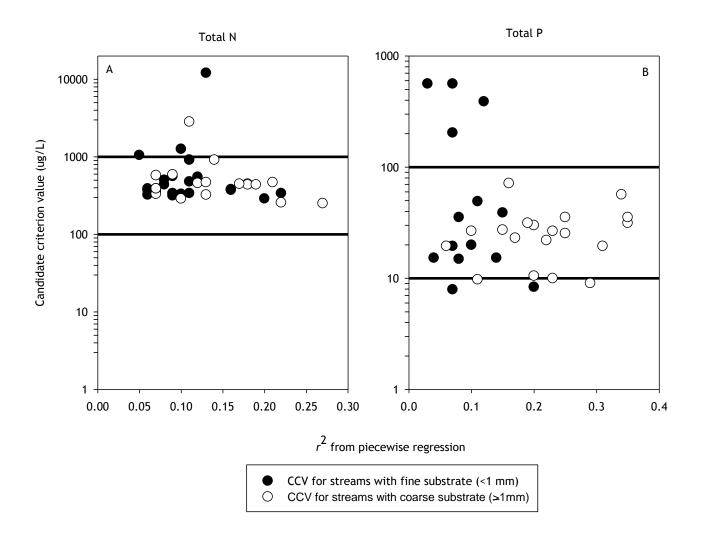


Fig. 8. Candidate threshold values (CTV) plotted against r^2 for the applicable piecewise regression (data from Tables 10 and 11). Horizontal lines at 100 and 1000 ug/L are for reference. CTVs < 1 not shown.

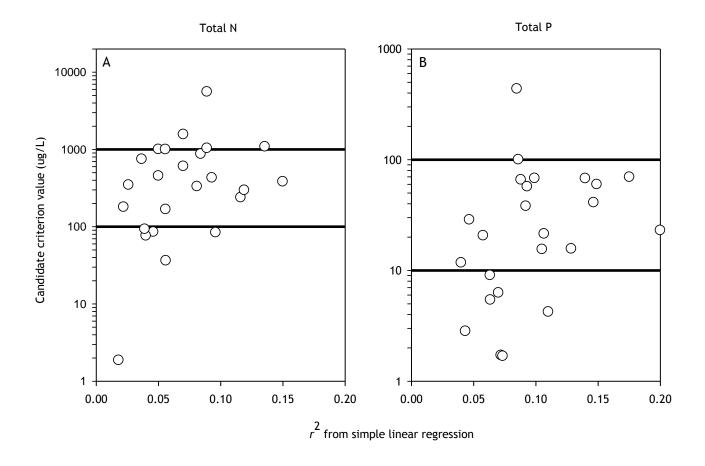


Fig. 9. Candidate threshold values (CTV) from interpolation plotted against r^2 (data from Tables 12-13). CTVs based on interpolation of median values at reference sites. Horizontal lines at 100 and 1000 ug/L are for reference. CTVs < 1 not shown.

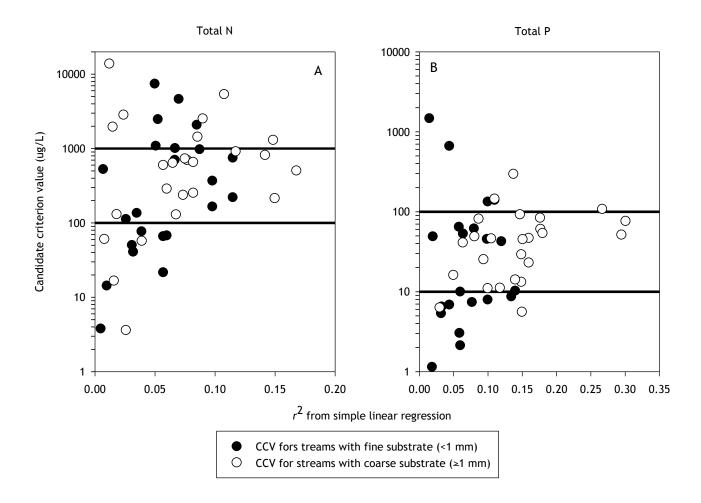


Fig. 10. Candidate threshold values (CTV) from interpolation plotted against r^2 (data from Tables 14-17). CTVs based on interpolation of median values at reference sites. Horizontal lines at 100 and 1000 ug/L are for reference. CTVs < 1 not shown.

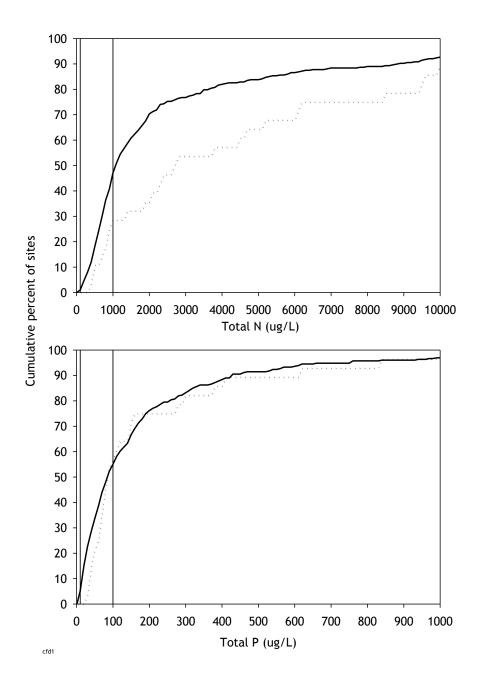


Figure 11. Cumulative percentage distributions of nutrient concentration. Values on the *y*-axis are the percent of all sites with a value equal to or less than the corresponding *x*-axis value. Solid lines are for all Plains/Upper Midwest sites; dotted line is for all sites in Illinois and Indiana. Vertical lines correspond to reference lines in Figs. 7-10.

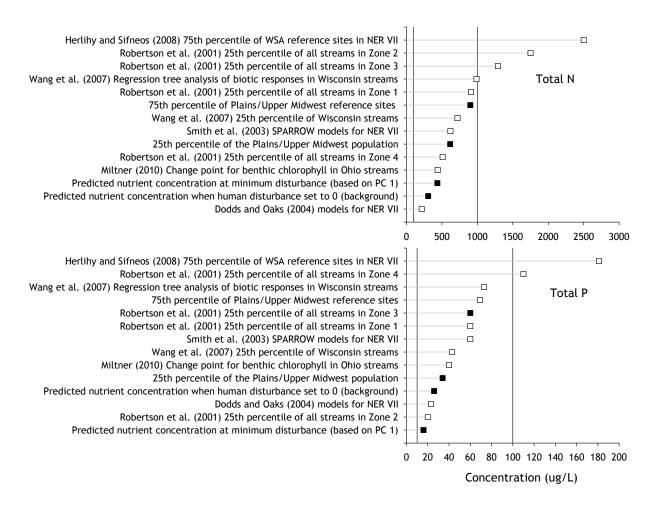


Figure 12. Nutrient candidate threshold values (CTV) for the Midwest. The Plains/Upper Midwest includes the following WSA aggregated ecoregions: Temperate Plains, Upper Midwest, Northern Plains, Southern Plains. EPA Nutrient Ecoregion (NER) VII is the "Corn Belt and Northern Great Plains." Illinois and Indiana are mostly in Robertson et al.'s N Zone 2 and mostly in P Zones 3 and 4. N criteria from Miltner (2010) are for DIN. Vertical reference lines correspond to lines in Fig. 11 and indicate the range of CTVs suggested by biotic responses (individual macroinvertebrate metrics) to nutrients. Filled symbols are from this report; open symbols are published values.

Appendix 1. Results of multiple linear models to predict metric values from natural factors and nutrients. Only factors with significant type III effects are shown. See Appendix 10 for variable definitions.

Sites	Metric	Lat. (dd)	Long. (dd)	Mean width (m)	Mean Slope (%)	Watershed Area (km²)	Precipitation (m/y)	Substrate (mm)	
		F based on type III SS $(p < 0.05)$							
All $(n = 327)$	OLLEPTAX	4.84		6.92				21.22	
	TL07RICH	9.45				9.82		18.3	
	HBI			13.38				45.88	
	PLECRICH	28.19			13.57		7.01	18.6	
	ODONRICH	14.93							
	CLMBRICH			4.01					
	TL89PTAX			5.34	9.35	4.24		20.67	
	TL01RICH	20.53			6.24		4.00	11.21	
	SHRDRICH	10.76			4.43	8.66			
	HABT_PT				19.87			22.22	
	TL67RICH			5.15		4.21			
	SCRPRICH	11.87	7.07					22.82	
	TOLR_PT	7.44			8.41	8.49	3.80	15.27	
	TL03PIND	12.04	4.32	25.39	5.46		11.38	25.19	
	MMI_WSABEST	8.60	6.09		10.48	5.8		34.64	
	CHIRRICH					5.88			
	PREDRICH					5.95		6.92	
	HPRIME	14.01					7.59	6.12	
	SPRLRICH	6.75	5.51	4.93		6.14			
	INTLRICH	22.76						26.66	
	FEED_PT				5.18			6.64	
	COGARICH	10.10				5.86	3.95		
	EPT_RICH	25.82		7.37				44.43	
	EPHE_PT	12.53	4.39	7.57		4.14		31.88	
Illinois and Indiana	НВІ							8.01	
(n = 28)	PLECRICH		10.36						
	TL89PTAX							7.45	
	HABT_PT							5.44	
	SCRPRICH		6.64						
	TOLR_PT							6.58	
	TL03PIND							7.07	
	MMI_WSABEST							4.56	
	PREDRICH		4.49						
	INTLRICH							5.85	
	EPT_RICH							5.57	

Appendix 2. Mean values for natural factors. See Appendix 10 for explanation of variables. WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

Sites (n)	Lat. (dd)	Long. (dd)	Mean width (m)	Mean Slope (%)	Watershed Area (km²)	Precipitation (m/y)	Substrate diameter (mm)	Wentworth name
All (327)	42.82	-69.56	8.02	0.72	1610	0.69	0.31	Medium sand
TPL (130)	42.39	-93.17	8.12	0.59	948	0.81	0.35	Medium sand
UMW (55)	44.81	-89.51	6.10	0.59	81	0.82	0.42	Medium sand
NPL (94)	45.32	-103.24	7.50	0.92	2492	0.45	0.17	Fine sand
SPL (48)	36.82	-100.72	10.94	0.83	3675	0.63	0.47	Medium sand
CBP (61)	41.89	-92.24	8.64	0.52	251	0.84	0.38	Medium sand
Not CBP (266)	43.03	-97.55	7.87	0.76	1940	0.65	0.29	Medium sand
Reference (50)	41.90	-97.22	7.08	0.96	319	0.72	1.78	Very coarse sand
Not Reference (277)	42.98	-96.44	8.18	0.67	1856	0.68	0.22	Fine sand
IN and IL (28)	40.23	-92.24	7.01	1.01	123	0.97	0.46	Medium sand
Not IN or IL (299)	43.06	-97.35	8.11	0.69	1757	0.66	0.30	Medium sand

Appendix 3. Significant (p<0.05) spearman rank correlations among natural stream and stream location variables. Longitude values are negative in WSA data.

Natural variable	Longitude	Latitude	Watershed	Precipitation	Substrate	
Transfer Farinoic	(W)	(N)	area	Treespitation	Buostrate	
Latitude (N)	-0.23					
Watershed area (km ²)	-0.59	0.25				
Precipitation (m/y)	0.82	-0.52	-0.64			
Mean log-transformed substrate diameter (mm)	0.12	-0.22		0.26		
Mean slope (%)	-0.22	0.11		-0.20	0.16	
Mean width (m)			0.60		0.21	

Appendix 4. Mean diameter of substrate in streams dominated by coarse or fine substrates. WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

	Stream	s with	coarse substr	rate (mean ≥ 1 mm)	Stream	s with	fine substrate	(mean <1 mm)
Sites	% of sites	n	Mean diameter (mm)	Wentworth name	% of sites	n	Mean diameter (mm)	Wentworth name
All	30	99	4.50	Pebble	70	228	0.10	Very fine sand
TPL	31	40	5.28	Pebble	69	90	0.10	Very fine sand
UMW	33	18	4.16	Pebble	67	37	0.13	Fine sand
NPL	21	20	3.33	Granule	79	74	0.08	Very fine sand
SPL	44	21	4.74	Pebble	56	27	0.08	Very fine sand
CDD	20	17	2.50	Granule	72	4.4	Λ 10	Fine sand
CBP	28	17	2.59		72	44	0.18	
Not CBP	31	82	5.05	Pebble	69	184	0.08	Very fine sand
Reference	62	31	5.50	Pebble	38	19	0.28	Medium sand
Not Reference	25	68	4.17	Pebble	75	209	0.09	Very fine sand
IN and IL	39	11	6.82	Pebble	61	17	0.08	Very fine sand
Not IN or IL	29	88	4.27	Pebble	71	211	0.10	Very fine sand

Appendix 5. Summary of interpretations of biotic responses to nutrient concentration (plots in Appendix 4). Rt = response threshold; Rm = response midpoint; St = secondary threshold; CTV = Candidate threshold value; NA = not applicable. In every case, the default CTV is at Rt based on the

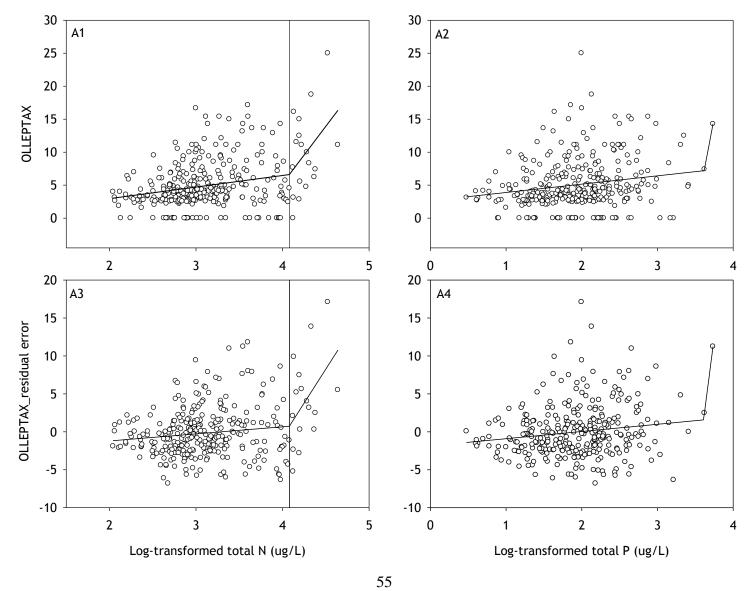
hypothesized responses in Fig. 3.

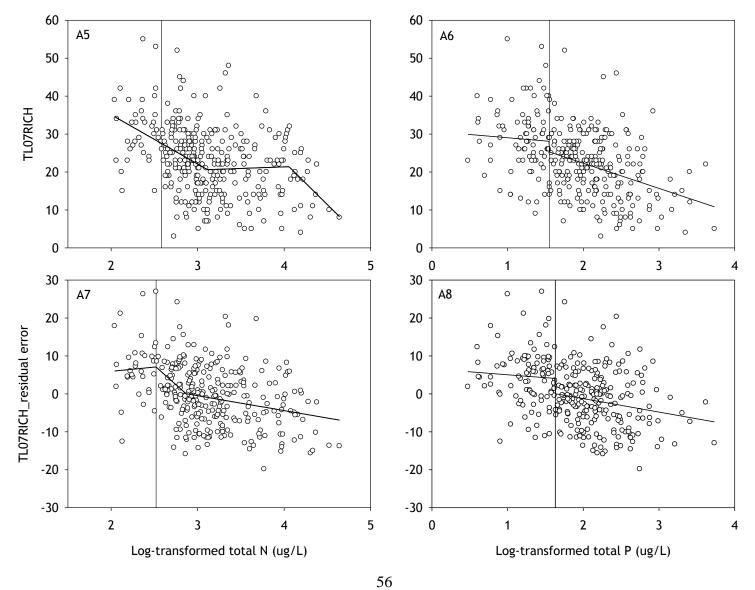
	hypothesized responses i	1119.3.	Plot type	
Plot	Metric	Nutrient	from Fig.	Interpretation of CTV from plot
A1	OLLEPTAX	Total N	F	Biotic response RT at high nutrient value; highly tolerant organisms
A2	OLLEPTAX	Total P	NA	Unreliable breakpoint at extreme of data
A3	OLLEPTAX_residual error	Total N	F	Biotic response RT at high nutrient value; highly tolerant organisms
A4	OLLEPTAX_residual error	Total P	NA	Unreliable breakpoint at extreme of data
A5	TL07RICH	Total N	С	Threshold set at midpoint of range of initial response.
A6	TL07RICH	Total P	A	Apparent change in biotic response at Rt; weak St
A7	TL07RICH_residual error	Total N	A	Threshold set at Rt; close match to hypothesized response
A8	TL07RICH_residual error	Total P	A	Apparent change in biotic response at Rt; weak St
A9	НВІ	Total N	В	Apparent change in biotic response at Rt; weak St
A10	НВІ	Total P	В	Suspect break point at high value
A11	HBI_residual error	Total N	В	Threshold set at Rt; close match to hypothesized response
A12	HBI_residual error	Total P	NA	Unreliable breakpoint at extreme of data
A13	PLECRICH	Total N	A	Threshold set at Rm because of high variability at extreme of data
A14	PLECRICH	Total P	G	Threshold set at midpoint of range of initial response.
A15	PLECRICH_residual error	Total N	G	Threshold set at midpoint of range of initial response.
A16	PLECRICH_residual error	Total P	A	Threshold set at Rt; close match to hypothesized response
A17	ODONRICH	Total N	A	Apparent change in biotic response at Rt; weak St
A18	ODONRICH	Total P	G	Threshold set at midpoint of range of initial response.
A19	ODONRICH_residual error	Total N	A	Threshold set at Rt; close match to hypothesized response
A20	ODONRICH_residual error	Total P	A	Apparent change in biotic response at Rt; weak St
A21	CLMBRICH	Total N	Е	Apparent change in biotic response at Rt; close match to hypothesized response
A22	CLMBRICH	Total P	Е	Apparent change in biotic response at Rt; close match to hypothesized response
A23	CLMBRICH_residual error	Total N	NA	Unreliable breakpoint at extreme of data
A24	CLMBRICH_residual error	Total P	A	Apparent change in biotic response at Rt; weak St
A25	TL89PTAX	Total N	Н	Threshold set at midpoint of range of initial response.
A26	TL89PTAX	Total P	NA	Unreliable breakpoint at extreme of data
A27	TL89PTAX_residual error	Total N	Н	Threshold set at <i>Rm</i> of range of initial response.
A28	TL89PTAX_residual error	Total P	Н	Threshold set at <i>Rm</i> mdpoint of range of initial response.
A29	TL01RICH	Total N	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A30	TL01RICH	Total P	A	Apparent change in biotic response at Rt; weak St
A31	TL01RICH_residual error	Total N	A	Threshold set at Rm because of high variability at extreme of data
A32	TL01RICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A33	SHRDRICH	Total N	С	Threshold set at midpoint of range of initial response.
A34	SHRDRICH	Total P	G	Threshold set at midpoint of range of initial response.

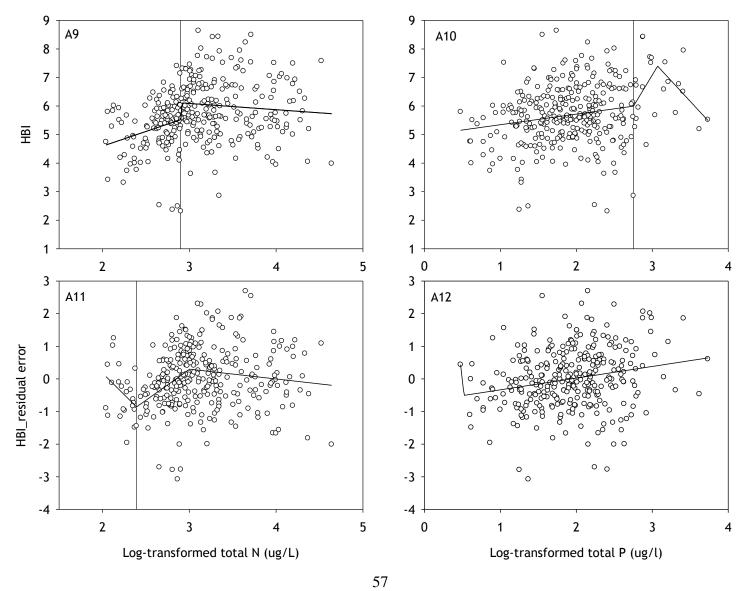
A35	SHRDRICH_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A36	SHRDRICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A37	HABT_PT	Total N	A	Threshold set at Rm because of high variability at extreme of data
A38	HABT_PT	Total P	С	Threshold set at midpoint of range of initial response.
A39	HABT_PT_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A40	HABT_PT_residual error	Total P	С	Threshold set at midpoint of range of initial response.
A41	TL67RICH	Total N	A	Apparent change in biotic response at <i>Rt</i> ; close match to hypothesized response
A42	TL67RICH	Total P	A	Apparent change in biotic response at <i>Rt</i> ; close match to hypothesized response
A43	TL67RICH_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A44	TL67RICH_residual error	Total P	A	Apparent change in biotic response at <i>Rt</i>
A45	SCRPRICH	Total N	G	Threshold set at midpoint of range of initial response.
A46	SCRPRICH	Total P	NA	Unreliable breakpoint at extreme of data
A47	SCRPRICH_residual error	Total N	NA	No breakpoint
A48	SCRPRICH_residual error	Total P	С	Threshold set at midpoint of range of initial response.
A49	TOLR_PT	Total N	С	Threshold set at midpoint of range of initial response.
A50	TOLR_PT	Total P	NA	Unreliable breakpoint at extreme of data
A51	TOLR_PT_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A52	TOLR_PT_residual error	Total P	NA	Unreliable breakpoint at extreme of data
A53	TL03PIND	Total N	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A54	TL03PIND	Total P	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A55	TL03PIND_residual error	Total N	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A56	TL03PIND_residual error	Total P	NA	Unreliable breakpoint at extreme of data
A57	MMI_WSABEST	Total N	С	Threshold set at midpoint of range of initial response.
A58	MMI_WSABEST	Total P	NA	No breakpoint
A59	MMI_WSABEST_residual error	Total N	NA	Unreliable breakpoint at extreme of data
1137	MMI_WSABEST_residual		1471	
A60	error	Total P	NA	No breakpoint
A61	CHIRRICH	Total N	С	Threshold set at midpoint of range of initial response.
A62	CHIRRICH	Total P	Е	Threshold set at <i>Rt</i> ; close match to hypothesized response
A63	CHIRRICH_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A64	CHIRRICH_residual error	Total P	Е	Threshold set at Rt; close match to hypothesized response
A65	PREDRICH	Total N	NA	Unreliable breakpoint at extreme of data
A66	PREDRICH	Total P	Е	Threshold set at Rt; close match to hypothesized response
A67	PREDRICH_residual error	Total N	Е	Threshold set at St because of high variability at extreme of data
A68	PREDRICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A69	HPRIME	Total N	С	Threshold set at midpoint of range of initial response.
A70	HPRIME	Total P	Е	Suspect breakpoint at high value (range of few data)
A71	HPRIME_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A72	HPRIME_residual error	Total P	Е	Suspect breakpoint at high value (range of few data)
A73	SPRLRICH	Total N	С	Threshold set at midpoint of range of initial response.

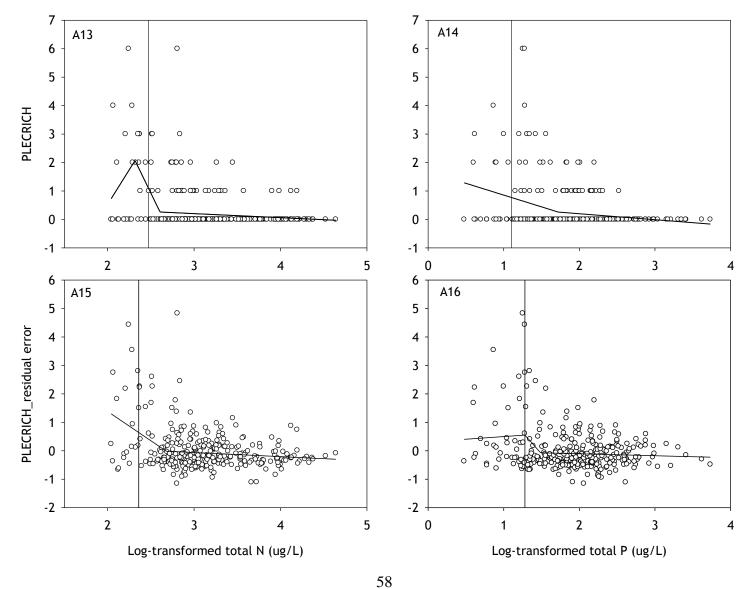
A74	SPRLRICH	Total P	Е	Threshold set at <i>Rt</i> ; close match to hypothesized response
A75	SPRLRICH_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A76	SPRLRICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A77	INTLRICH	Total N	A	Threshold set at Rm because of high variability at extreme of data
A78	INTLRICH	Total P	G	Threshold set at midpoint of range of initial response.
A79	INTLRICH_residual error	Total N	A	Threshold set at Rm because of high variability at extreme of data
A80	INTLRICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A81	FEED_PT	Total N	A	Threshold set at Rm because of high variability at extreme of data
A82	FEED_PT	Total P	A	Apparent change in biotic response at Rt; weak St
A83	FEED_PT_residual error	Total N	A	Apparent change in biotic response at Rt; weak St
A84	FEED_PT_residual error	Total P	A	Apparent change in biotic response at Rt; weak St
A85	COGARICH	Total N	С	Threshold set at midpoint of range of initial response.
A86	COGARICH	Total P	E	Threshold set at Rt; close match to hypothesized response
A87	COGARICH_residual error	Total N	С	Threshold set at midpoint of range of initial response.
A88	COGARICH_residual error	Total P	Е	Threshold set at Rt; close match to hypothesized response
A89	EPT_RICH	Total N	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A90	EPT_RICH	Total P	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A91	EPT_RICH_residual error	Total N	A	Threshold set at <i>Rm</i> because of high variability at extreme of data
A92	EPT_RICH_residual error	Total P	G	Threshold set at midpoint of range of initial response.
A93	EPHE_PT	Total N	A	Apparent change in biotic response at Rt; weak St
A94	EPHE_PT	Total P	G	Threshold set at midpoint of range of initial response.
A95	EPHE_PT_residual error	Total N	NA	Unreliable breakpoint at extreme of data
A96	EPHE_PT_residual error	Total P	NA	No breakpoint

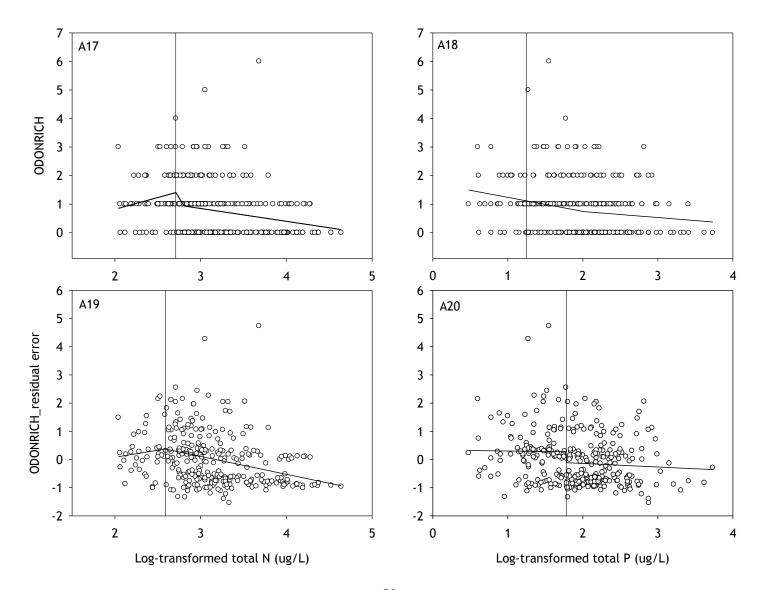
Appendix 6. Plots of relationships between nutrient concentration and raw macroinvertebrate metrics and between nutrient concentration and residual error from natural variation models. Vertical line is the CTV from Tables 9 and 10.

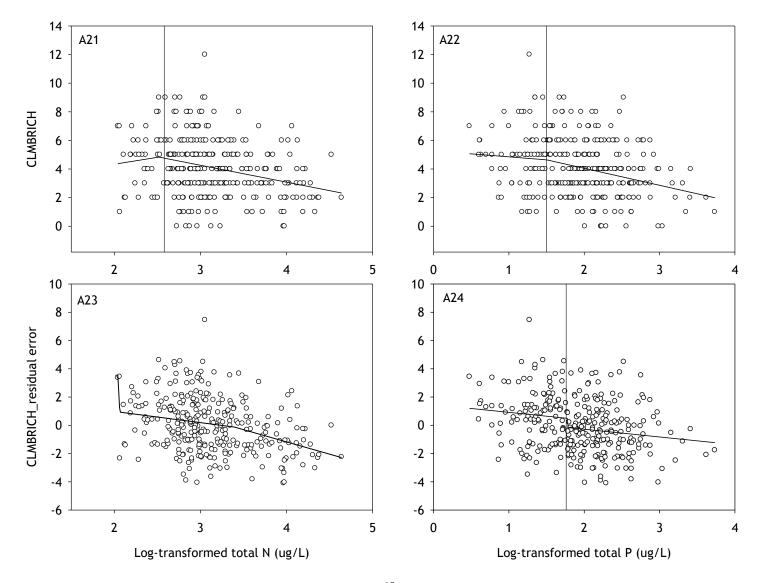


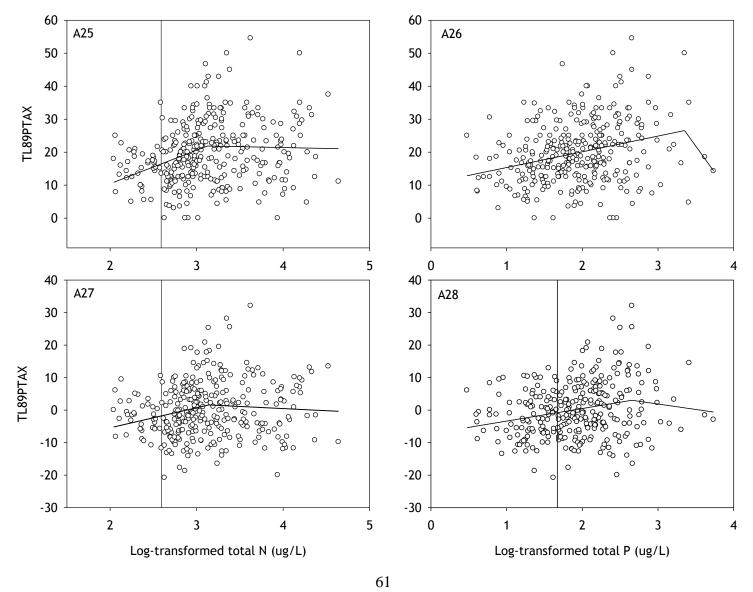


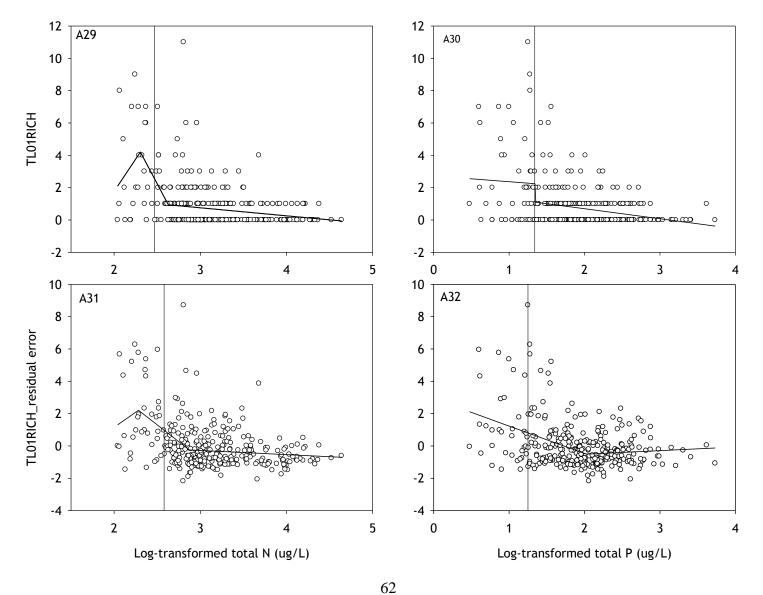


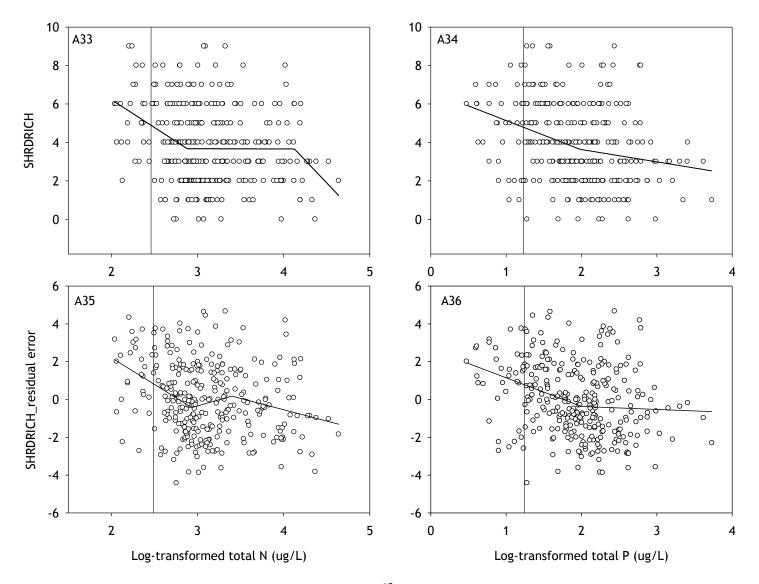


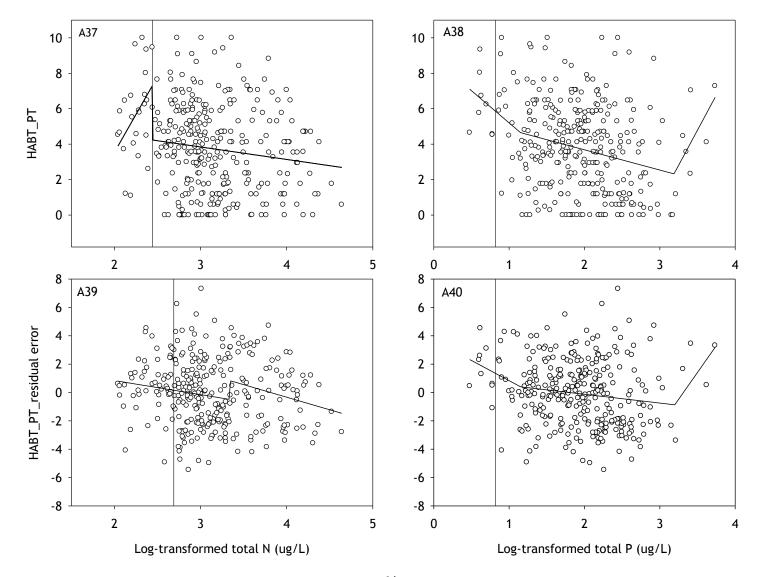


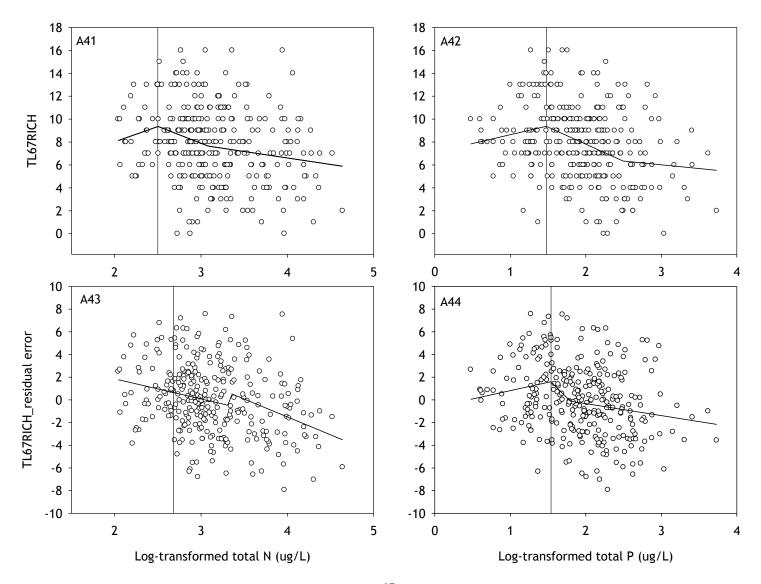


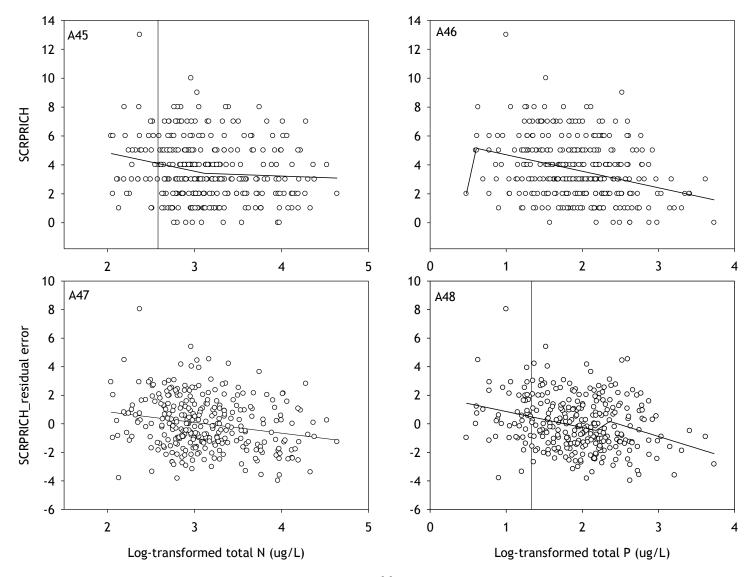


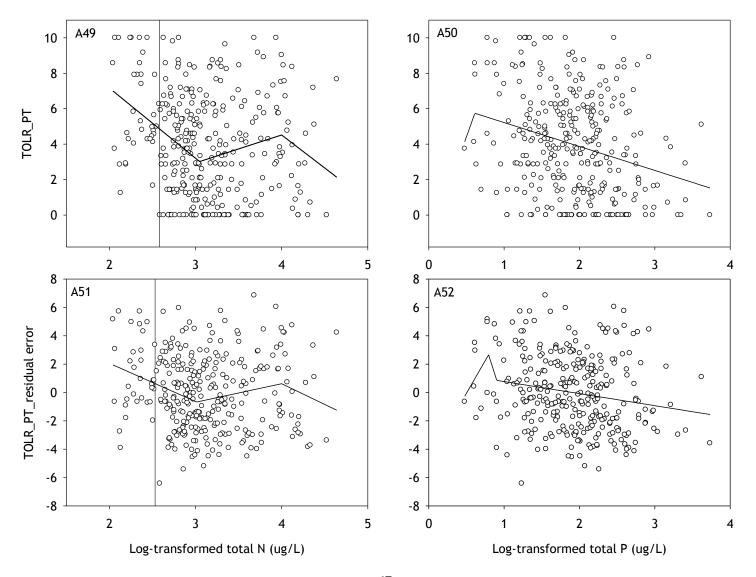


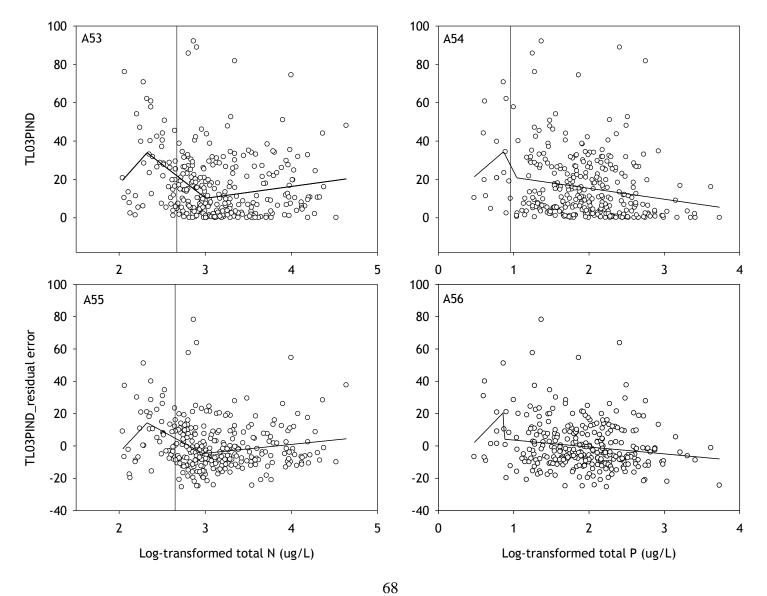


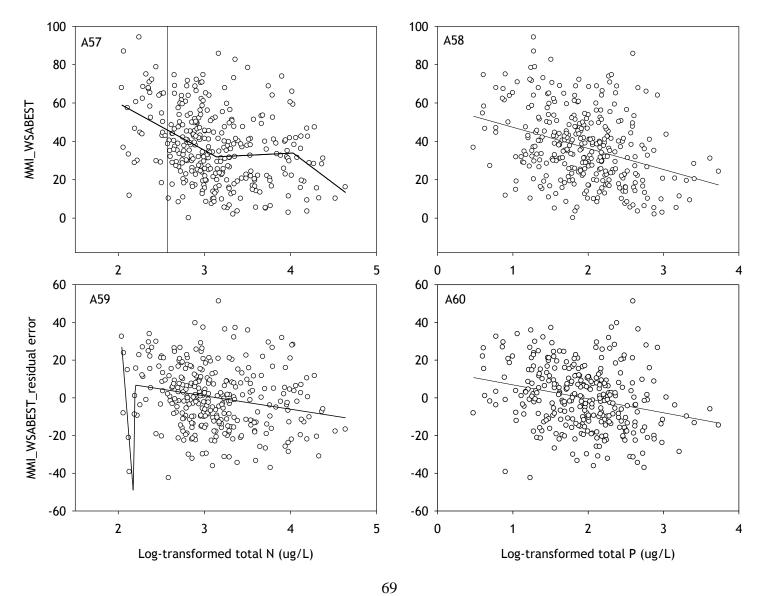


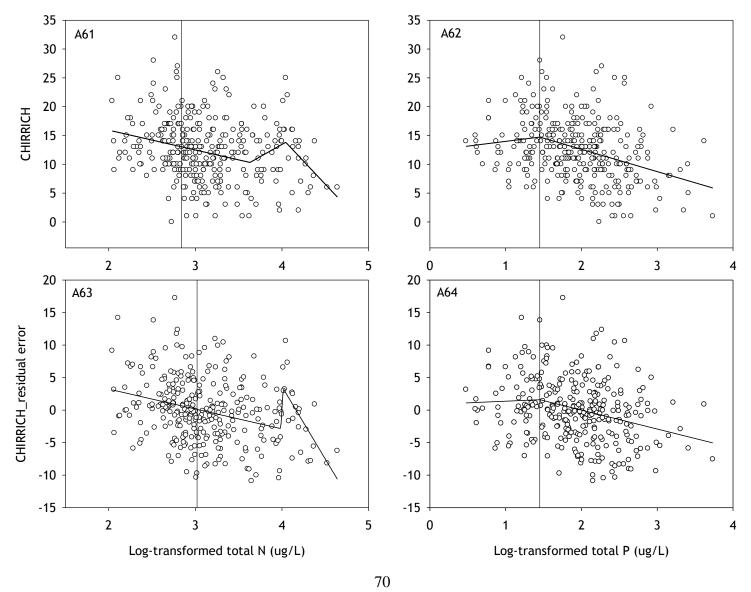


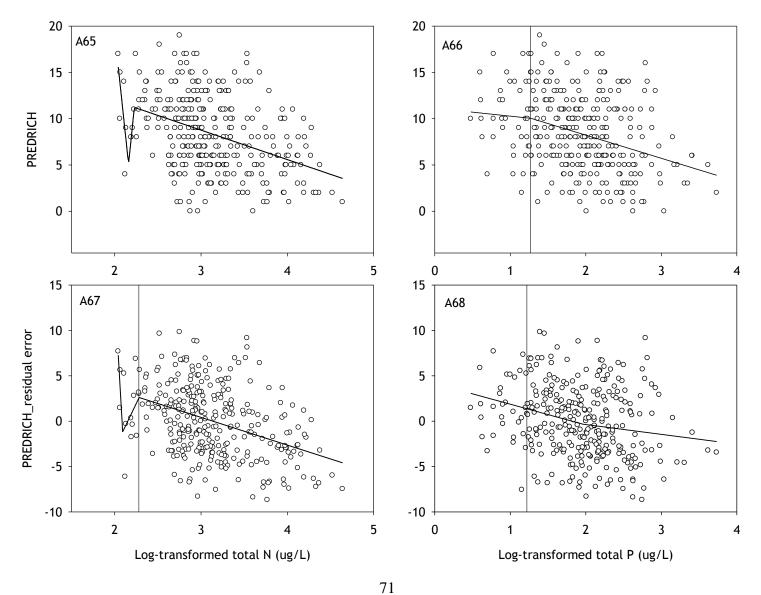


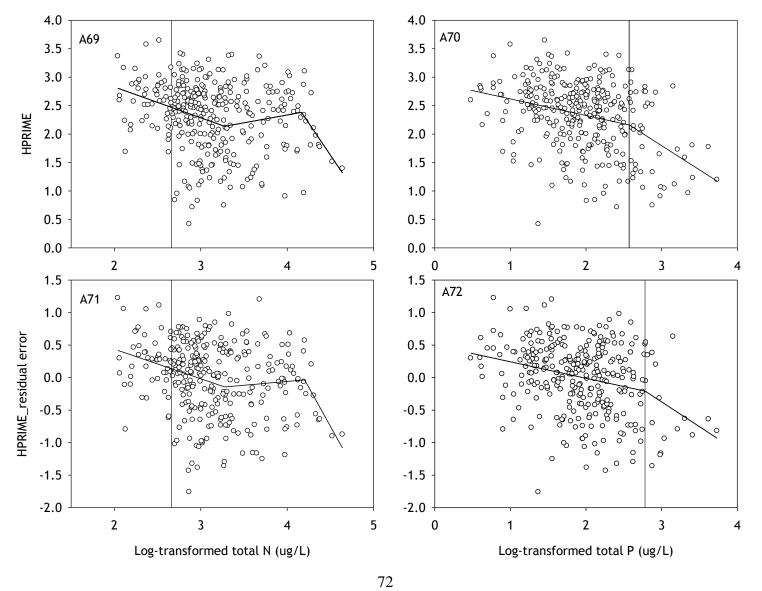


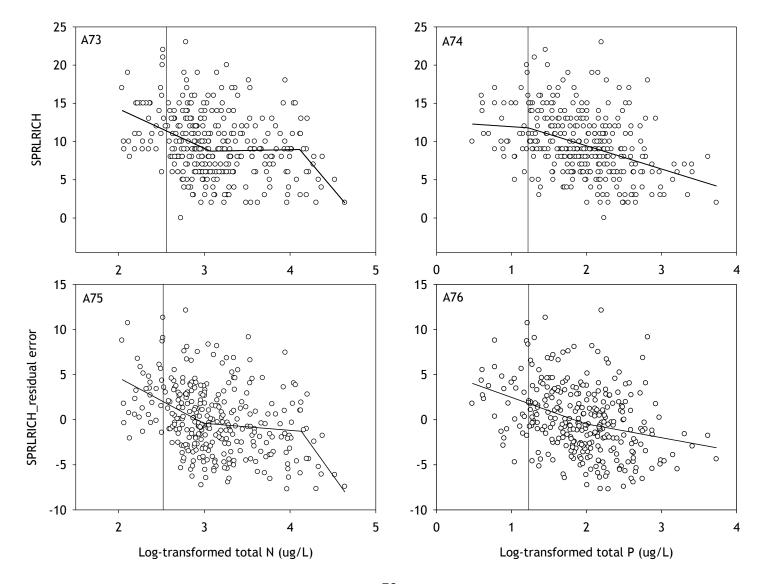


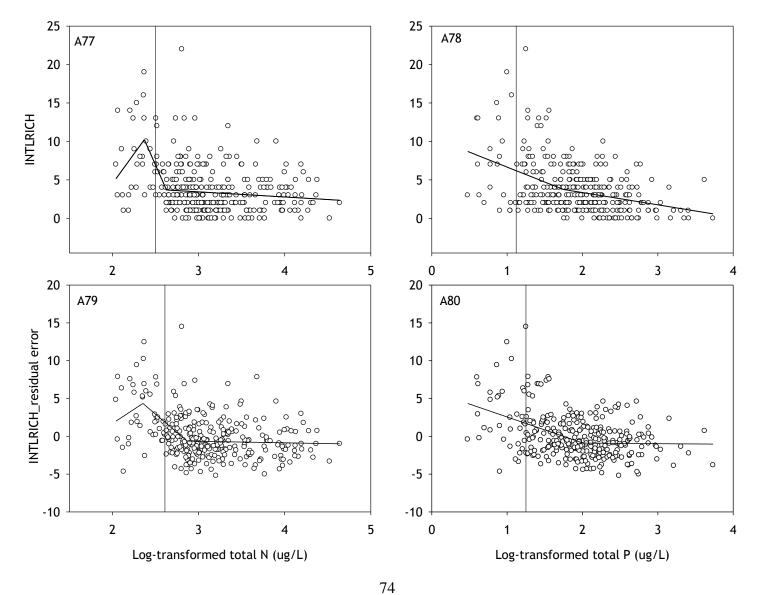


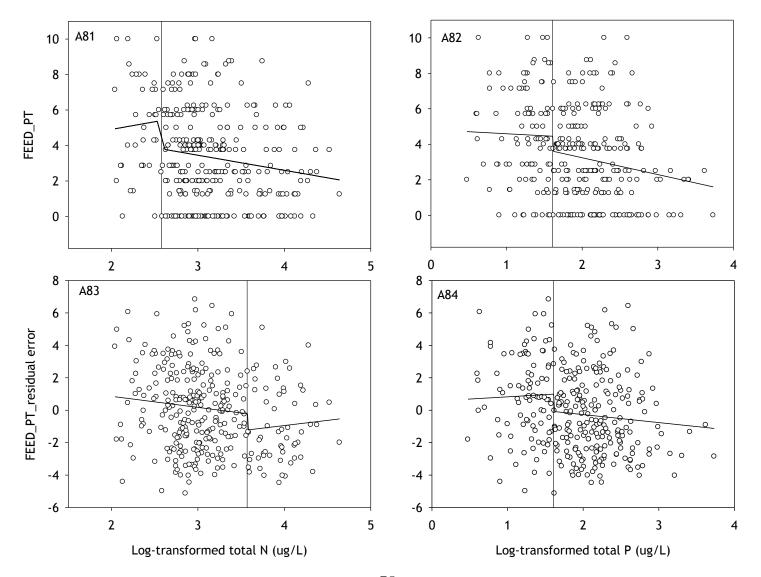


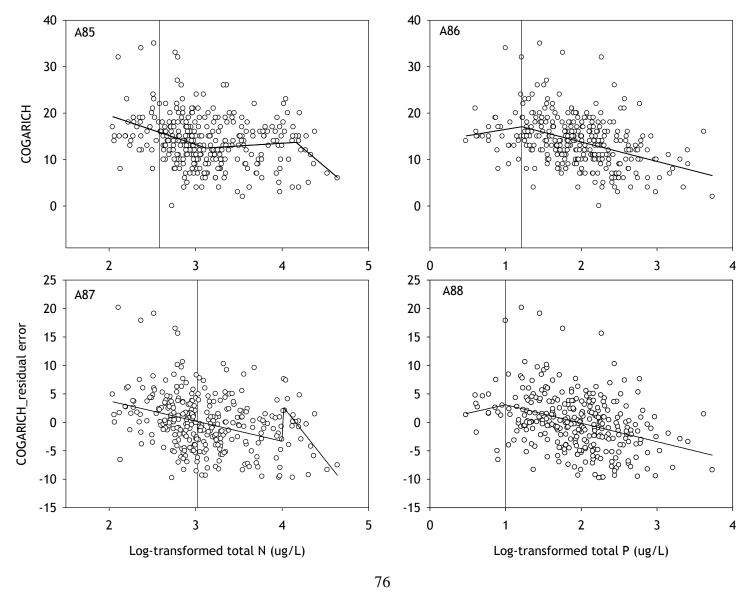


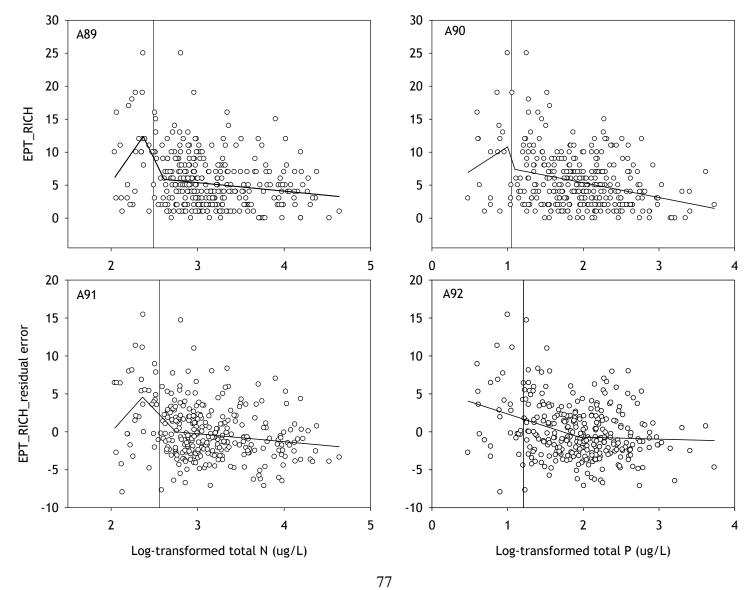


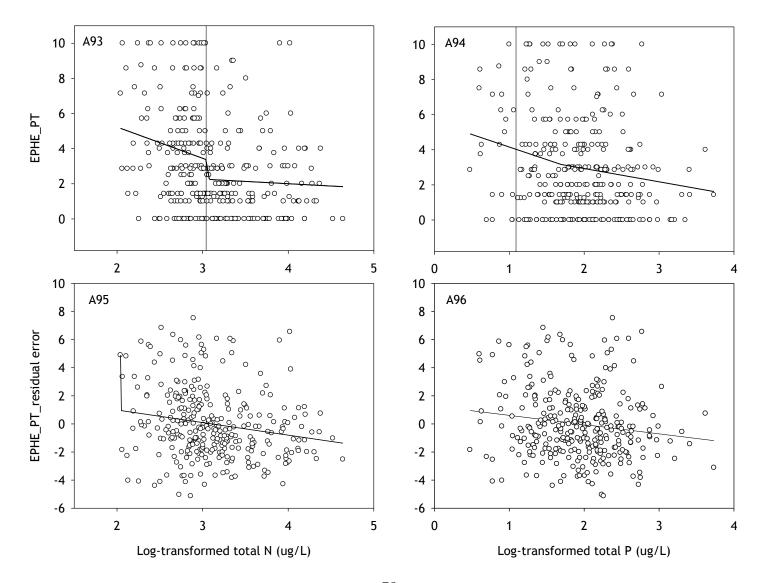




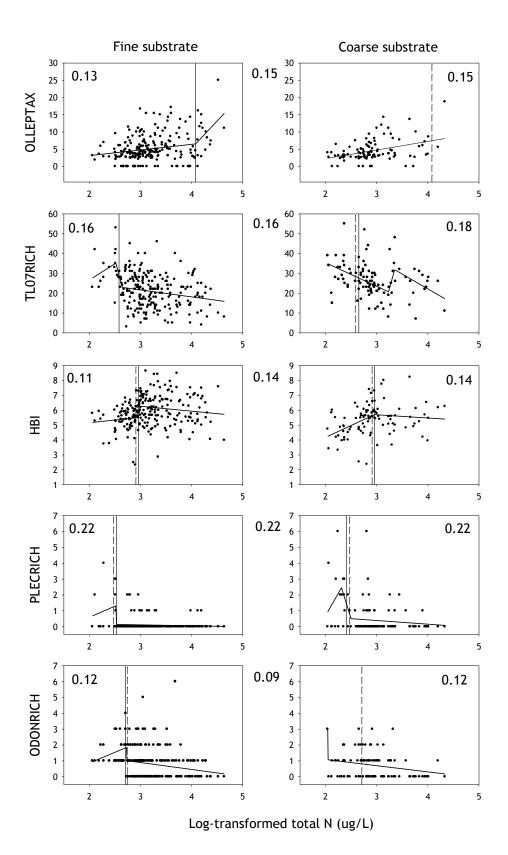


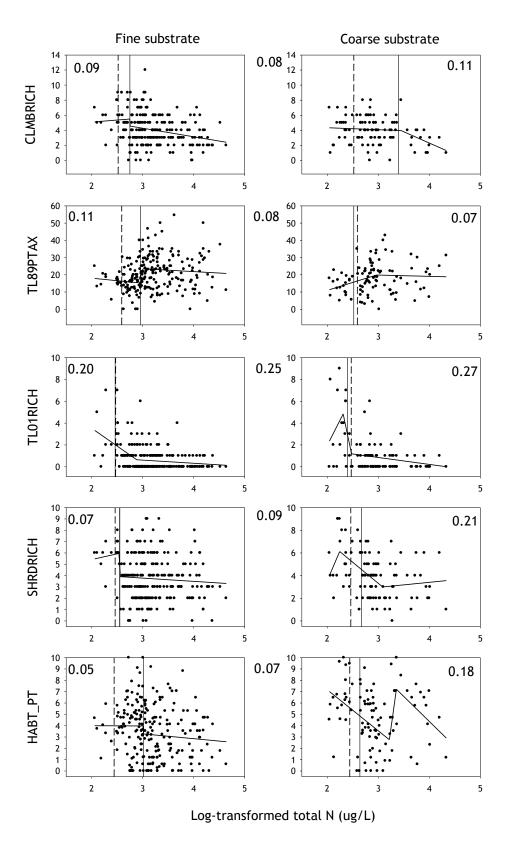


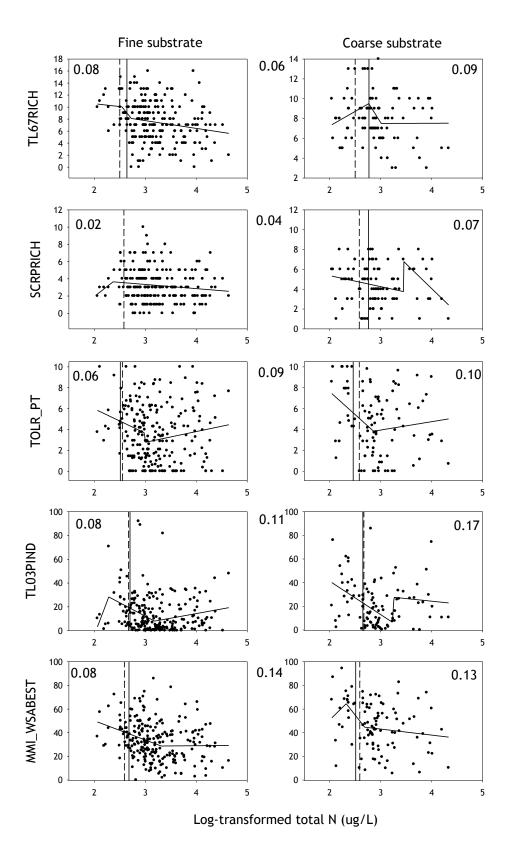


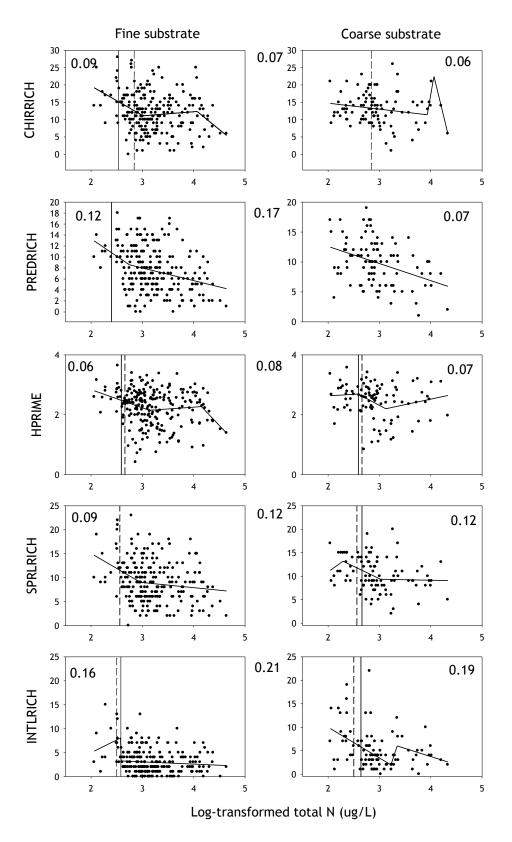


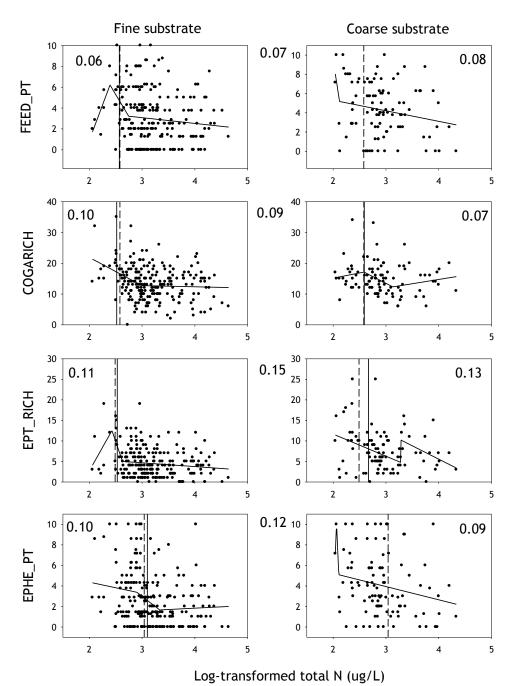
Appendix 7. Plots of relationships between nutrient concentration and raw macroinvertebrate metrics for streams with fine substrate (<1 mm) and streams with coarse substrate (≥1 mm). Dashed vertical lines are CTVs for all streams for comparison. Solid vertical lines are CTVs from Tables 10 and 11; r^2 values show for each metric on plot; r^2 values between plots are for all streams for comparison. The rationale for identifying CTV is the same as described in Appendix 3.

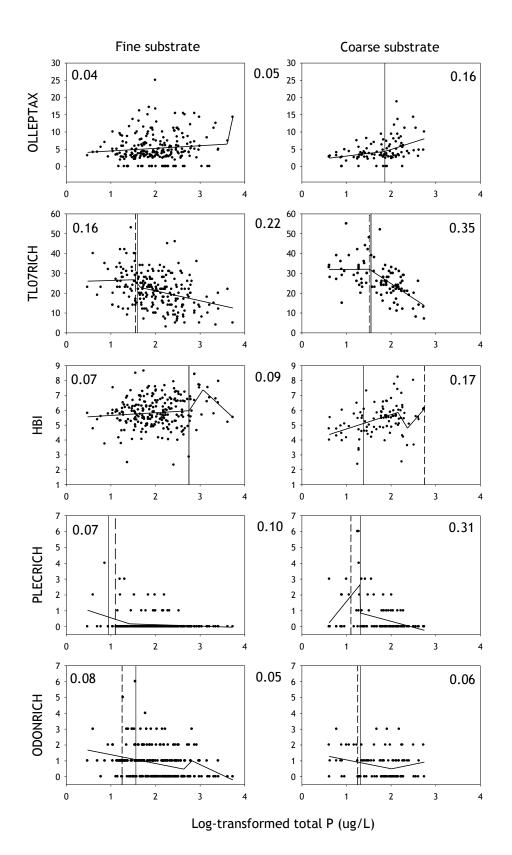


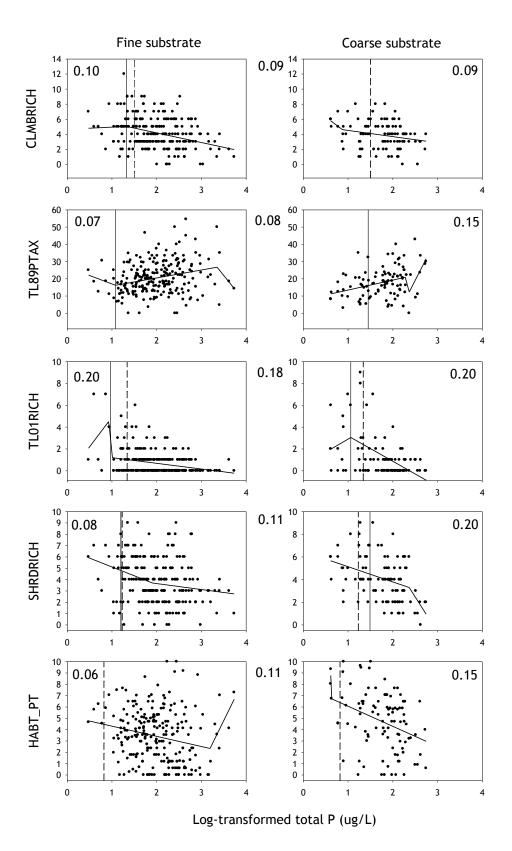


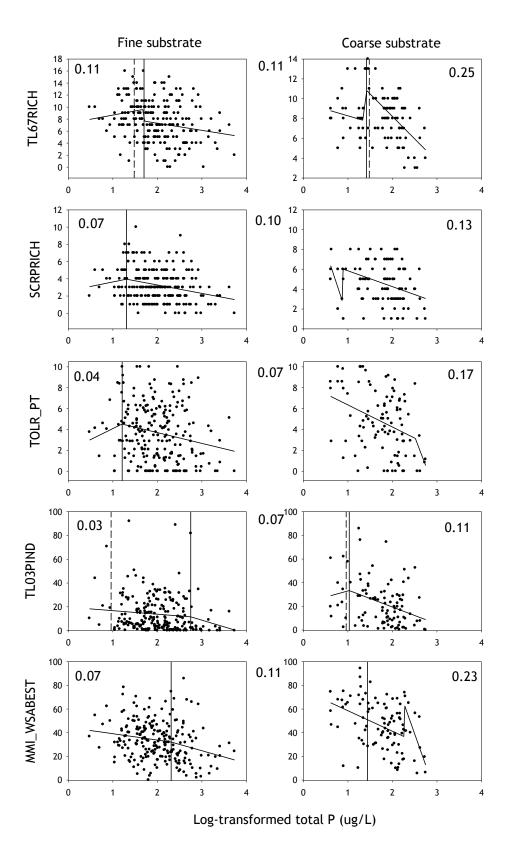


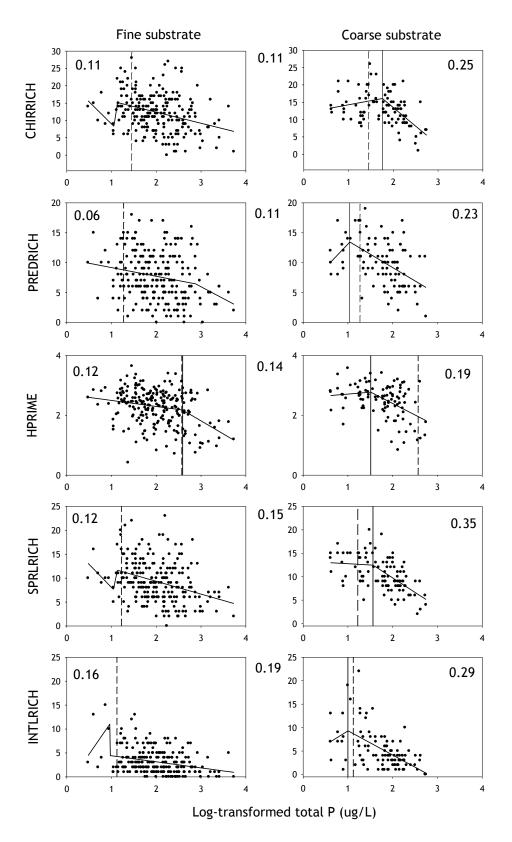


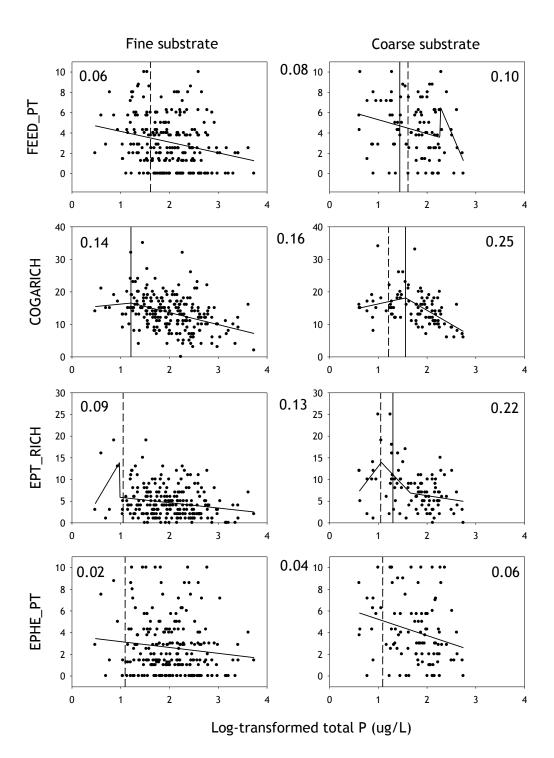




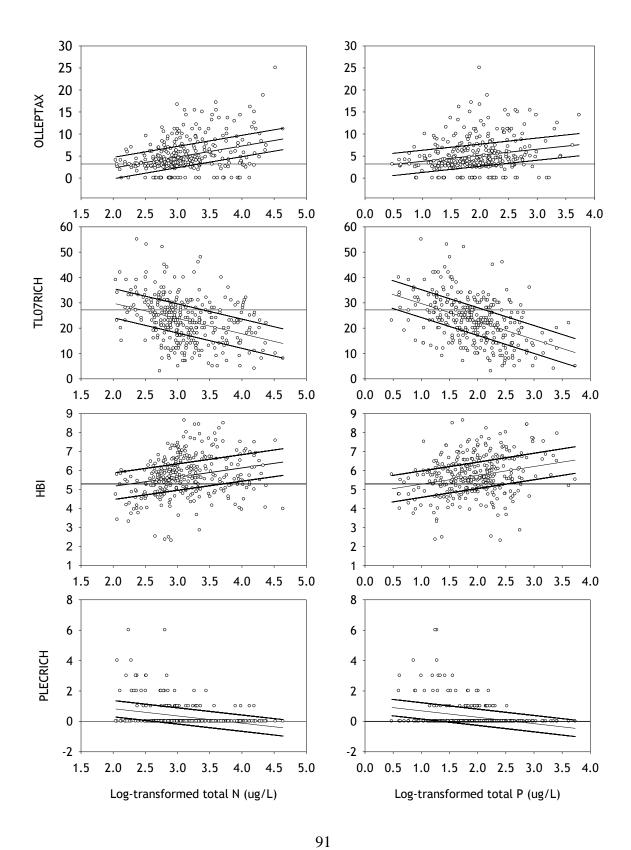


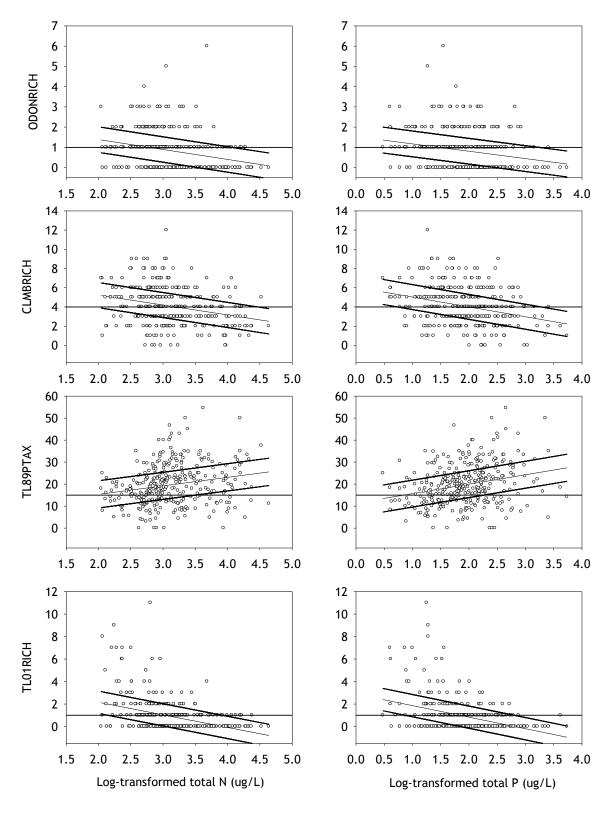


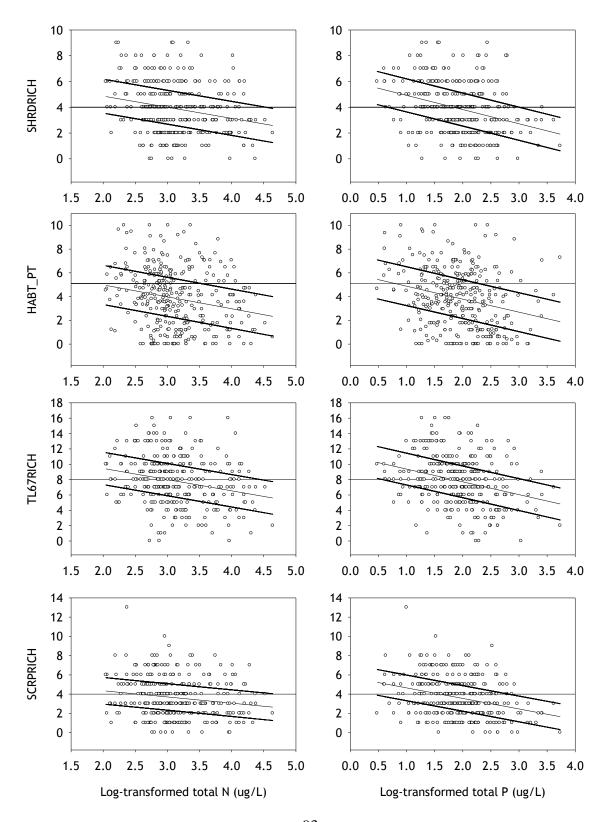


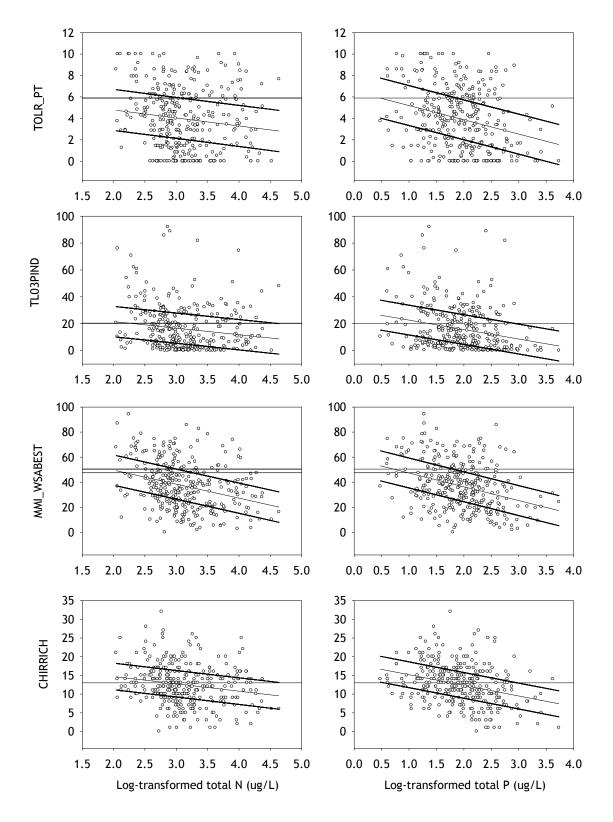


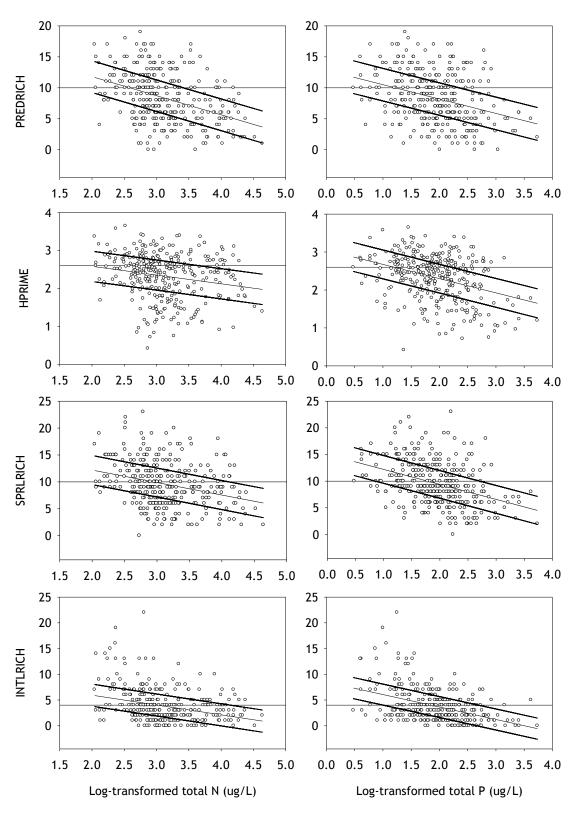
Appendix 8. Plots of simple linear regressions with 50% prediction intervals between nutrient concentration and raw macroinvertebrate metrics. Horizontal lines are median values from reference sites. Details in Tables 12-13.



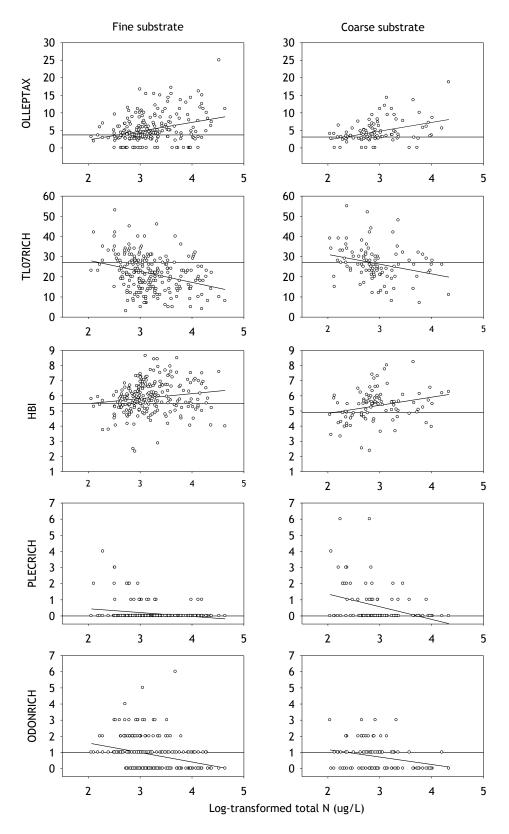


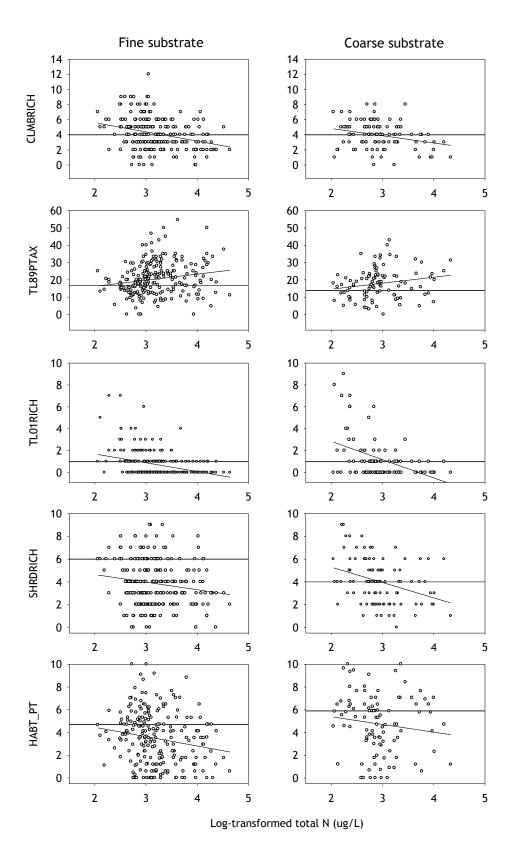


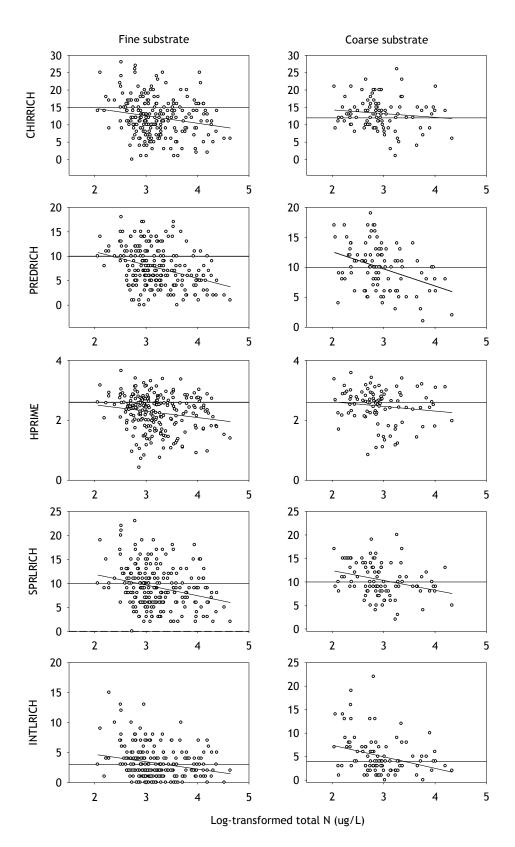


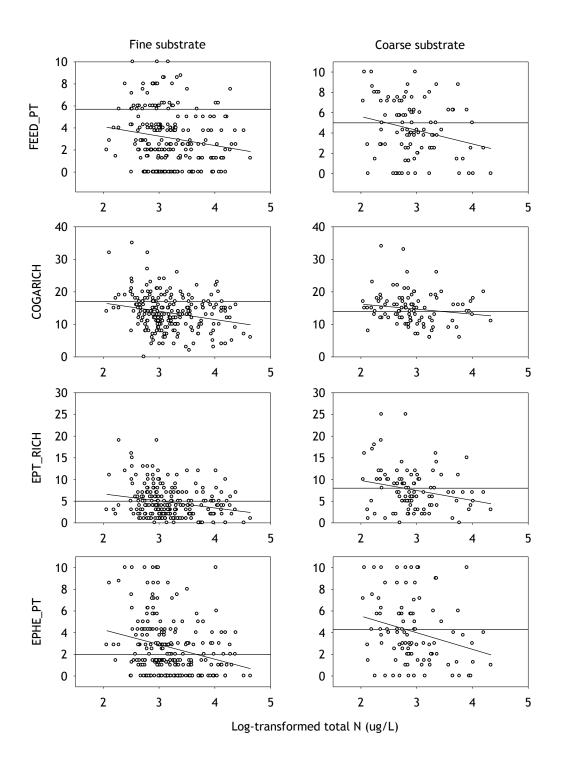


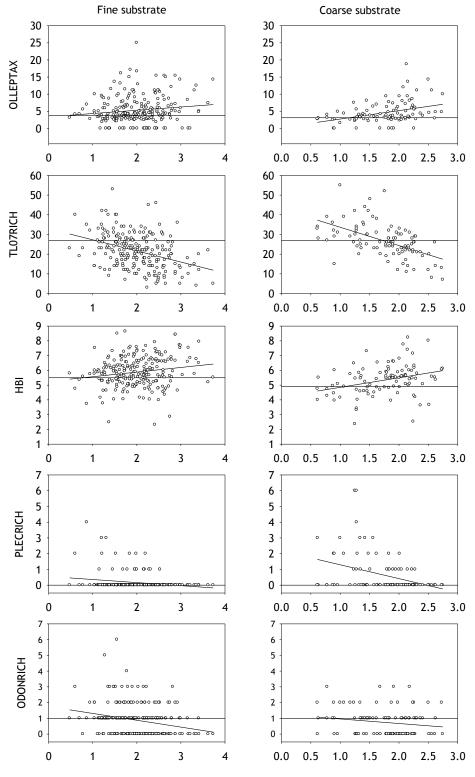
Appendix 9. Plots of simple linear regressions with between nutrient concentration and raw macroinvertebrate metrics for streams with fine and coarse substrate. Horizontal lines are median values from reference sites. Details in Tables 14-17.



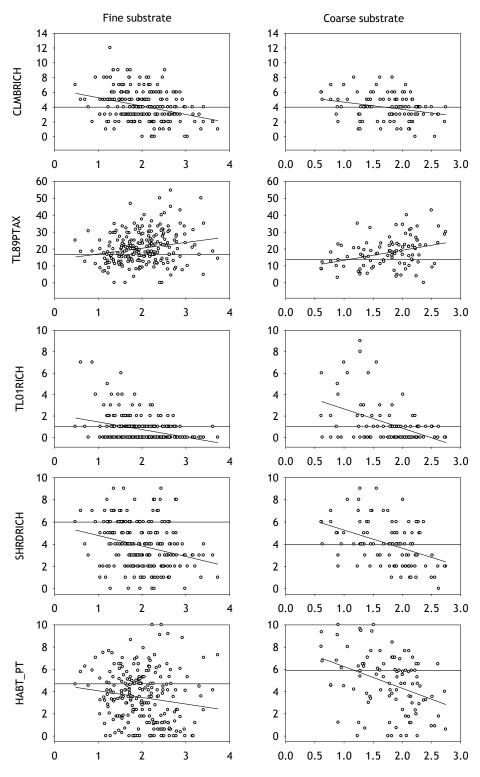




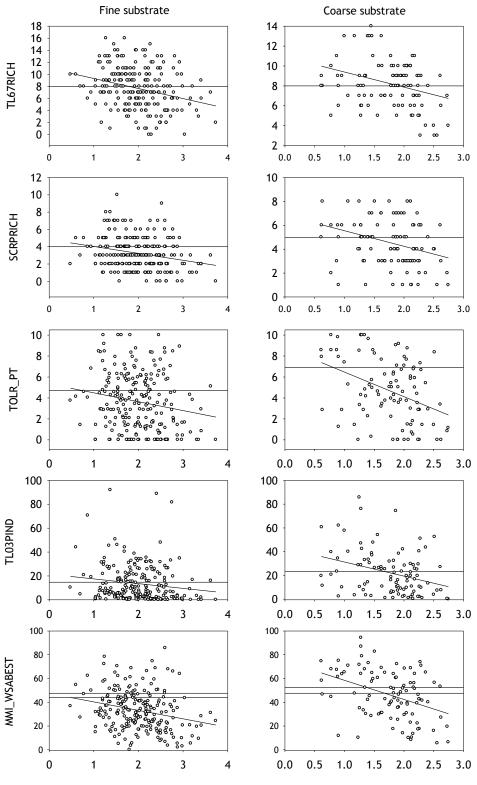




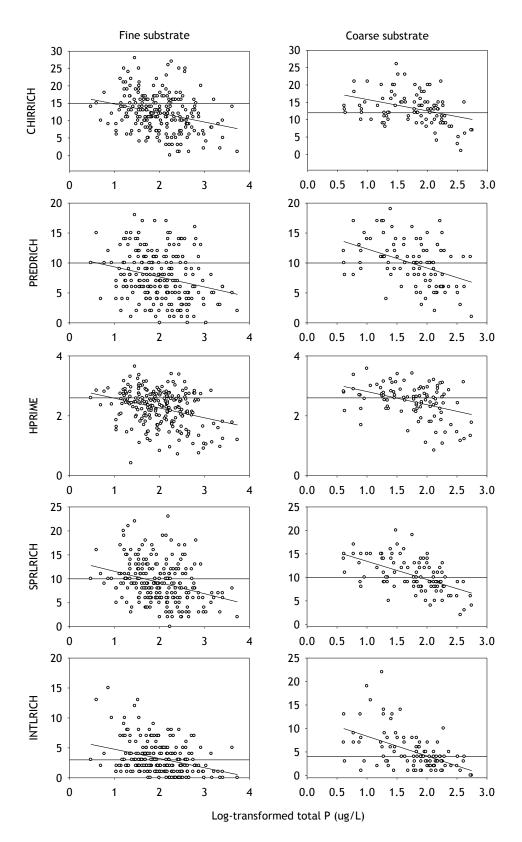
 $Log\text{-}transformed\ total\ P\ (ug/L)$

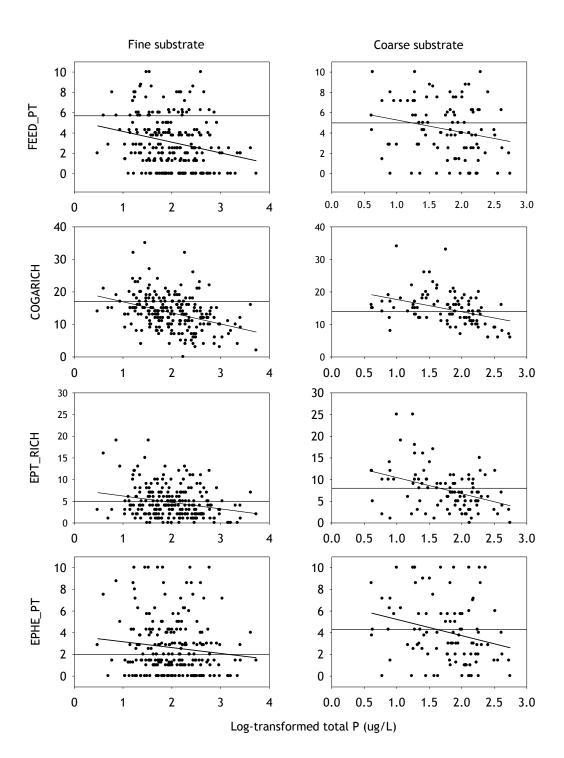


Log-transformed total P (ug/L)



Log-transformed total P (ug/L)





Appendix 10. Predictive and response variables used in this report.

Variable	Explanation	Source
PREDRICH	Predator Distinct Taxa Richness	WSA
TL07RICH	PTV 0-7.9 Distinct Taxa Richness	WSA
TL01RICH	Percent tolerance value = 0-1.9 Distinct Taxa Richness	WSA
EPHE_PT	Richness Metric Score for Nationwide MMI based on Ephemeroptera richness	WSA
ODONRICH	Odonata Distinct Taxa Richness	WSA
MMI_WSABEST	Best 6 WSA MMI	WSA
SPRLRICH	Sprawler Distinct Taxa Richness	WSA
INTLRICH	Intolerant Distinct Taxa Richness	WSA
CLMBRICH	Climber Distinct Taxa Richness	WSA
EPT_RICH	EPT Distinct Taxa Richness	WSA
PLECRICH	Plecoptera Distinct Taxa Richness	WSA
TL67RICH	PTV 6-7.9 Distinct Taxa Richness	WSA
FEED_PT	FFG Best 6 MMI Scoring	WSA
COGARICH	Collector-Gatherer Distinct Taxa Richness	WSA
HPRIME	Shannon Diversity	WSA
SHRDRICH	Shredder Distinct Taxa Richness	WSA
HABT_PT	Habit Best 6 MMI Scoring	WSA
CHIRRICH	Chironomid Distinct Taxa Richness	WSA
TL03PIND	PTV 0-3.9 % Individuals	WSA
SCRPRICH	Scraper Distinct Taxa Richness	WSA
TOLR_PT	Tolerance Best 6 MMI Scoring	WSA
TL89PTAX	PTV 8-10 % Distinct Taxa	WSA
HBI	Hilsenhoff Biotic Index	WSA
OLLEPTAX	Oligochaete/Leech % Distinct Taxa	WSA
% row crops	NLCD class 82 "cultivated crops"	NLCD 2001
% pasture/hay	NLCD class 81"pasture/hay"	NLCD 2001
% developed	Sum of NLCD classes 21-24 "developed open space, low, medium and high intensity"	NLCD 2001
% forest	Sum of NLCD classes 41-43 "deciduous, evergreen and mixed forest"	NLCD 2001
% wetlands	Sum of NLCD classes 90 and 95 "woody wetlands, emergent herbaceous wetlands"	NLCD 2001
Population density	1990 Human population density (WSA variable name POPDENS)	WSA
Road density	Road density (WSA variable name RDDENS)	WSA
Riparian disturbance	Proximity weighted index based on presence of human disturbance (WSA variable name W1_HALL)	WSA
Riparian canopy openness	100 – mean stream bank canopy density (WSA variable name XCDENBK)	WSA
Mean width	Mean channel width in m (WSA variable name XWIDTH)	WSA
Mean slope	Mean channel width in m (WSA variable name XSLOPE)	WSA
Watershed area	Watershed area (WS area) in km2 (WSA variable name LANDAREA)	WSA
Precipitation	Annual precipitation in m (WSA variable name PRECIP_M	WSA
Substrate	Log-transformed mean diameter of substrate (WSA variable name LSUB_DMM)	WSA
Latitude	WSA variable name LAT_DD	WSA
Longitude	WSA variable name LON_DD	WSA

Appendix 11. Tables 10 - 14 with all parameters back transformed. CTVs for total N based on interpretation of piecewise regression models. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by "(+)". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

Metric		Pie	cewise model	interpretation bas	ed on raw met	Piecewise model interpretation based on regression residuals								
	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)
OLLEPTAX (+)	0.15		12022	43651	22908	4.08	12022	0.12		12022	43651	22908	4.08	12022
TL07RICH	0.16	109	1287		375	2.58	379	0.18		330	740	494	2.52	330
HBI (+)	0.14		793	831	812	2.91	812	0.09		244	1022	500	2.39	244
PLECRICH	0.22		208	406	291	2.47	294	0.14	109		478	228	2.36	228
ODONRICH	0.09		512	645	574	2.71	512	0.10		388	43651	4120	2.59	388
CLMBRICH	0.08		330	43651	3801	2.52	330	0.12		2454	43651	10350		
TL89PTAX (+)	0.08	109		1348	384	2.59	388	0.03	109		1379	388	2.59	388
TL01RICH	0.25		199	426	291	2.47	294	0.21		190	740	375	2.58	379
SHRDRICH	0.09	109	758		287	2.46	287	0.09	109		850	304	2.49	308
HABT_PT	0.07		274	274	274	2.44	274	0.04	109		2137	483	2.69	489
TL67RICH	0.06		315	1201	616	2.50	315	0.08	109		2041	472	2.68	478
SCRPRICH	0.04	109	1287		375	2.58	379	0.04	109		43651	2187		
TOLR_PT	0.09	109	1121		350	2.55	354	0.05	109		1022	334	2.53	338
TL03PIND	0.11		208	1022	461	2.67	467	0.09		208	932	441	2.65	446
MMI_WSABEST	0.14	109	1379		388	2.59	388	0.07		147	157	152		
CHIRRICH	0.07	109	4265		683	2.84	691	0.11	109		9771	1034	3.02	1046
PREDRICH	0.17		144	169	156			0.16		122	190	152	2.28	190
HPRIME	0.08	109		1904	456	2.66	456	0.14	109		1904	456	2.66	456
SPRLRICH	0.12	109		1174	358	2.56	362	0.16	109		976	326	2.52	330
INTLRICH	0.21		233	426	315	2.50	315	0.21		223	740	406	2.61	406
FEED_PT	0.07		338	416	375	2.58	379	0.04		3714	3889	3801	3.57	3714
COGARICH	0.09	109		1317	379	2.58	379	0.12	109		9999	1046	3.02	1046
EPT_RICH	0.15		233	406	308	2.49	308	0.13		228	561	358	2.56	362
EPHE_PT	0.12		1095	1201	1147	3.04	1095	0.05	109		43651	2187		
Median	0.10						379	0.11						388

Appendix 11, continued. CTVs for total P based on interpretation of piecewise regression models. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, St = maximum nutrient value, St = nutrient valu

Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by "(+)". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

	P	iecewise 1	model int	erpretation	n based o	on raw me	Piecewise model interpretation based on regression residuals							
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)
OLLEPTAX (+)	0.05	2		5369	126			0.06	2		5369	126		
TL07RICH	0.22		34	35	35	1.55	34	0.17		42	42	42	1.63	42
HBI (+)	0.09		561	1174	812	2.75	561	0.05	2		5369	126		
PLECRICH	0.10	2		51	12	1.10	12	0.10		18	30	18	1.39	24
ODONRICH	0.05	2		101	17	1.25	17	0.06		59	59	59	1.78	59
CLMBRICH	0.09		31	5369	411	1.50	31	0.10		53	61	57	1.76	57
TL89PTAX (+)	0.08	2		5369	126			0.05	2		456	36	1.57	36
TL01RICH	0.18		21	21	21	1.34	21	0.16	2		104	17	1.25	17
SHRDRICH	0.11	2		94	16	1.23	16	0.09	2		99	16	1.24	16
HABT_PT	0.10	2		13	6	0.82	6	0.05	2		13	6	0.82	6
TL67RICH	0.11		29	308	96	1.48	29	0.09		34	61	45	1.54	34
SCRPRICH	0.10	2		5369	126			0.07	2	150		20	1.33	20
TOLR_PT	0.07	2		5369	126			0.05		5	7	6	0.9	7
TL03PIND	0.07		6	10	8	0.96	8	0.06		6	7	6		
MMI_WSABEST	0.11	2		5369	126			0.06	2		5369	126		
CHIRRICH	0.11		27	5369	388	1.45	27	0.07		27	5369	388	1.45	27
PREDRICH	0.11		18	5369	315	1.27	18	0.06	2		90	16	1.22	16
HPRIME	0.14		371	5369	1412	2.57	371	0.10		602	5369	1798	2.78	602
SPRLRICH	0.15		16	43651	850	1.22	16	0.12	2		94	16	1.23	16
INTLRICH	0.19	2		55	12	1.12	12	0.17	2		101	17	1.25	17
FEED_PT	0.08		40	41	40	1.61	40	0.06		40	41	40	1.61	40
COGARICH	0.16		15	5369	294	1.21	15	0.12		9	5369	231	1	9
EPT_RICH	0.13		9	12	10	1.05	10	0.09	2		86	15	1.21	15
EPHE_PT	0.04	2		48	11	1.09	11	0.02	2		5369	126		
Median	0.11						17	0.07						20

Appendix 11, continued. CTVs for total N based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. Mn = minimum observed nutrient concentration, Rt = response threshold, St = secondary threshold, Mx = maximum nutrient value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used

to calculate *Rm*. Missing values for CTV are relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by "(+)". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

S not materi exactly an			el interpre	etation bas	ed on rav	w metrics	Piecewise model interpretation base on raw metrics for streams with coarse substrates (≥1mm)							
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)
OLLEPTAX (+)	0.13		12022	21379	16031	4.08	12022	0.15	114			114		
TL07RICH	0.16		330	416	371	2.57	371	0.18	114	1697		441	2.65	446
HBI (+)	0.11		793	1022	901	2.96	911	0.14		911	43651	6309	2.96	911
PLECRICH	0.22		330	338	334	2.53	338	0.22		203	315	253	2.41	256
ODONRICH	0.12		536	549	542	2.74	549	0.12		111	43651	2212		
CLMBRICH	0.09		561	561	561	2.75	561	0.11		2817	43651	11091	3.45	2817
TL89PTAX (+)	0.11		793	1022	901	2.96	911	0.07	114	932		326	2.52	330
TL01RICH	0.20	109	758		287	2.46	287	0.27		203	301	247	2.40	250
SHRDRICH	0.07		346	362	354	2.55	354	0.21		173	1229	461	2.67	467
HABT_PT	0.05		1022	1046	1034	3.02	1046	0.18	114	1659		436	2.64	436
TL67RICH	0.08		346	536	431	2.64	436	0.09		588	1022	775	2.77	588
SCRPRICH	0.02		233	21379	2238			0.07	114	2817		568	2.76	574
TOLR_PT	0.06	109	954		323	2.51	323	0.10	114	723		287	2.46	287
TL03PIND	0.08		190	1317	500	2.70	500	0.17	114	1697		441	2.65	446
MMI_WSABEST	0.11	109	2041		472	2.68	478	0.13		208	489	319	2.51	323
CHIRRICH	0.09	109	1022		334	2.53	338	0.06	114	8510		988		
PREDRICH	0.12	109	561		247			0.07	114			114		
HPRIME	0.06	109	1348		384	2.59	388	0.07		388	1317	715	2.59	388
SPRLRICH	0.09	109	911		315	2.50	315	0.12		194	1046	451	2.66	456
INTLRICH	0.16		379	379	379	2.58	379	0.19	114	1659		436	2.64	436
FEED_PT	0.06		244	561	371	2.57	371	0.08	114	131		122		
COGARICH	0.10	109	999		330	2.52	330	0.07		388	1348	723	2.59	388
EPT_RICH	0.11		268	426	338	2.53	338	0.13	114	1904		467	2.67	467
EPHE_PT	0.10		793	1994	1258	3.10	1258	0.09	114	128		121		
Median	0.10						384	0.12						441

Appendix 11, continued. CTVs for total P based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. Mn = minimum observed nutrient concentration, Rt = minimum response threshold, St = minimum substrate size. St = minimum observed nutrient value. See Fig. 3 and text for explanation of terms. St = minimum observed nutrient value.

to calculate *Rm*. Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by "(+)". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

	Pie		nodel inter			on raw me	trics for	Piecewise model interpretation base on raw metrics for streams with coarse substrates (>1mm)							
Metric	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	r^2	Mn	Rt	St or Mx	Rm	CTV	CTV (ug/L)	
OLLEPTAX (+)	0.13	2	4073		110			0.16		71	549	199	1.86	71	
TL07RICH	0.15		32	46	38	1.60	39	0.35		31	549	132	1.51	31	
HBI (+)	0.07		561	1147	803	2.75	561	0.17	3	140		23	1.38	23	
PLECRICH	0.07	2	25		8	0.95	8	0.31		19	19	19	1.31	19	
ODONRICH	0.08	2	426		35	1.56	35	0.06	3	101		19	1.31	19	
CLMBRICH	0.10		20	5369	334	1.32	20	0.09	3	6		4			
TL89PTAX (+)	0.07		11	2238	163			0.15	3	190		27	1.45	27	
TL01RICH	0.20		8	9	8	0.97	8	0.20		10	549	78	1.06	10	
SHRDRICH	0.08	2	80		15	1.20	15	0.20	3	233		30	1.49	30	
HABT_PT	0.06	2	1548		67			0.15	3	3		3			
TL67RICH	0.11		46	51	49	1.70	49	0.25		20	25	23	1.42	25	
SCRPRICH	0.07		19	5369	330	1.31	19	0.13		6	7	7			
TOLR_PT	0.04		15	5369	294	1.21	15	0.17		323	549	421			
TL03PIND	0.03		561	5369	1737	2.75	561	0.11		10	549	76	1.03	10	
MMI_WSABEST	0.07		203	5369	1046	2.31	203	0.23	3	181		26	1.44	27	
CHIRRICH	0.11		10	12	11			0.34		57	549	177	1.76	57	
PREDRICH	0.06		758	5369	2017			0.23		10	549	77	1.04	10	
HPRIME	0.12		388	5369	1444	2.59	388	0.19		31	549	132	1.51	31	
SPRLRICH	0.12		10	12	11			0.35		35	549	140	1.56	35	
INTLRICH	0.16		8	8	8			0.29		9	549	73	1.00	9	
FEED_PT	0.06	2		5369	126			0.10	3	181		26	1.44	27	
COGARICH	0.14		15	5369	294	1.21	15	0.25		35	549	140	1.56	35	
EPT_RICH	0.09		9	9	9			0.22		10	45	22	1.36	22	
EPHE_PT	0.02	2		5369	24			0.06	3		549	46			
Median	0.08						28	0.20						27	

Appendix 12: Curve for back transforming nutrient concentrations.

